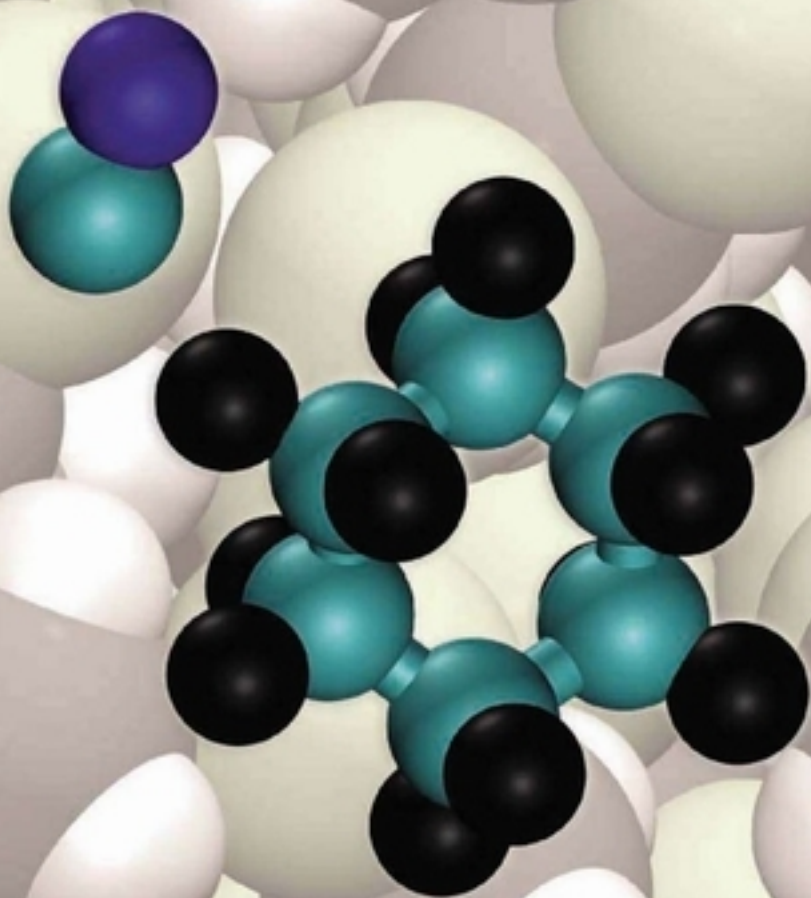


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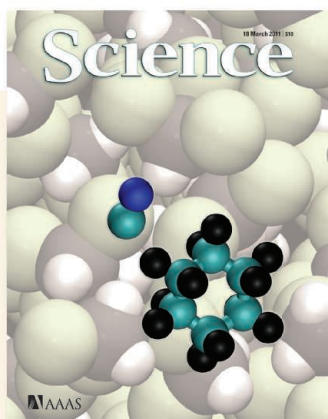
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COVER

Reaction of a CN radical with cyclohexane (ball-and-stick structures in green-blue and green-black, respectively) in dichloromethane solvent (space-filling representation). Observations of this reaction on the time scale of picoseconds reveal a wealth of information about the chemical mechanism and shed light on how solvents widely used in chemical synthesis influence reactions on a molecular scale. See page 1423.

Image: David R. Glowacki/School of Chemistry, University of Bristol

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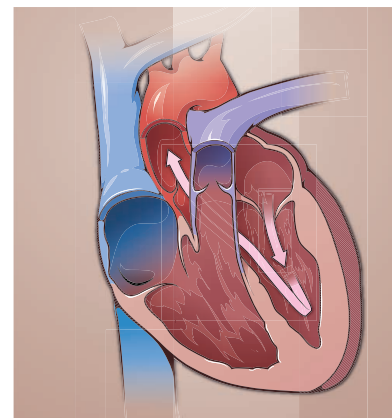
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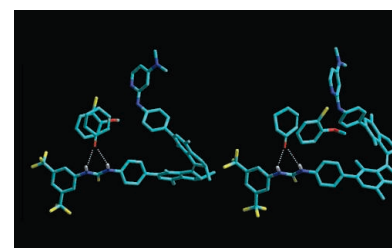
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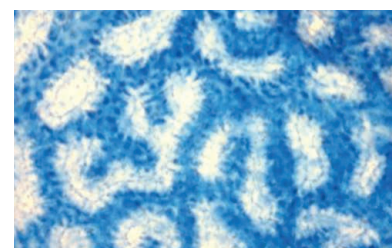
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SCIENCEEXPRESS

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The Hot Summer of 2010: Redrawing the Temperature Record Map of Europe

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10.1126/science.1201224

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Asteroseismic observations with the Kepler satellite probed the deep interior of an evolved star.
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Amino acid modulation of Ca²⁺ signaling guides growth of plant pollen tubes.
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Full text at www.sciencemag.org/cgi/content/full/331/6023/1387-c

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Getting to the Guts of Malnutrition

Gut bacteria may be an important factor in the response to starvation.
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Gut bacteria help mice survive potentially lethal infections.
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The Signal Transduction Knowledge Environment
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RESEARCH RESOURCE: Engineering the ABA Plant Stress Pathway for Regulation of Induced Proximity

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PERSPECTIVE: Inducible Gene Expression in Mammals—Plants Add to the Menu
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K. T. G. Rigbolt et al.

Dynamic phosphorylation during stem cell differentiation may control recruitment of DNA methyltransferases to silence genes that maintain pluripotency.

PODCAST

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SCIENCE CAREERS

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Free Career Resources for Scientists

Tooling Up: Making the Cut in 2011

D. Jensen

In a job market where even entry-level jobs are going to people with industry experience, how can you compete?

<http://scim.ag/toolingup2011>

Hunting Ghosts

S. Gaidos

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<http://scim.ag/huntingghosts>

SCIENCE TRANSLATIONAL MEDICINE

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Integrating Medicine and Science

16 March issue: <http://scim.ag/stm031611>

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RESEARCH ARTICLE: β -Arrestin Deficiency Protects Against Pulmonary Fibrosis in Mice and Prevents Fibroblast Invasion of Extracellular Matrix

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L. S. Ostedgaard et al.

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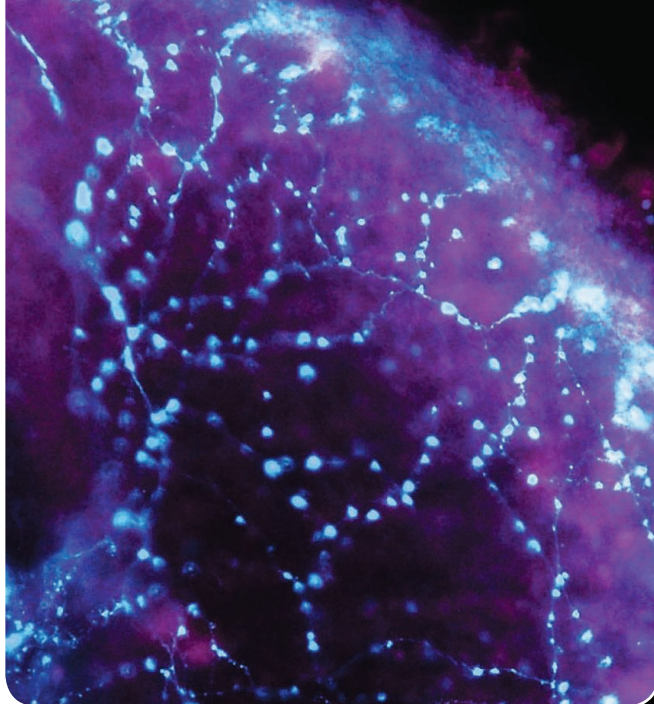
Science Policy News and Analysis

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ADVANCING SCIENCE. SERVING SOCIETY



<< The Brain's Alarm Clock

Circadian rhythms are linked to the external light-dark cycle through inputs from photoreceptors that signal into networks that regulate neural and physiological function. One of the key photoreceptors is CRYPTOCHROME, which is sensitive to blue-light wavelengths. **Fogle *et al.*** (p. 1409, published online 3 March; see the Perspective by **Im and Taghert**) now find that CRYPTOCHROME has an unexpectedly direct effect on circadian physiology in fruit flies. A small group of neurons that are part of the circadian circuit and that are usually more active in the morning express CRYPTOCHROME. These neurons normally receive plenty of input from the circadian circuit that perceives cycles and drives responses. However, when those inputs are blocked, it seems that these neurons are able to respond directly to blue light.

Moral Determinism?

Experimental philosophers have recently begun to ask fundamental, but seemingly straightforward, questions about the human condition.

Nichols (p. 1401) reviews the problem that free will, which is accepted as a given by most people, poses for determinism: Does free will absolve people of moral responsibility for their actions? Although a definitive resolution of this issue is not in sight, the experimental findings suggest explanations for why people behave as they do, even if the underlying motivations for their reasoning are still debated.

Gassing Up

Large igneous provinces (LIPs) are extremely large geological features formed by episodes of rapid and extensive volcanism, which, in the Late Triassic/Early Jurassic (about 200 thousand years ago), were often associated with massive extinction events. It is believed that LIP volcanism can have dramatic impacts on the composition of the atmosphere, including its CO₂ burden. Using evidence from soil carbonates, **Schaller *et al.*** (p. 1404, published online 17 February) reconstructed variations in the concentration of atmospheric CO₂ at the time of the emplacement of the Central Atlantic Magmatic Province of the Late Triassic/Early Jurassic. The data confirm that geological events can affect climate and thus drive large global extinctions.

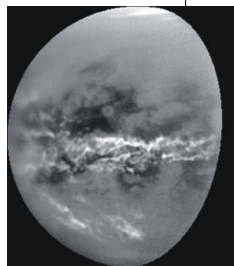
Chroming Earth's Core

Earth originally had a bulk composition not unlike meteorites, and comparing the composition of meteorites to Earth's differentiated layers—the

metallic core and silicate-rich mantle—reveals clues about Earth's formation. For example, why are elements like chromium, which are moderately volatile, depleted in the mantle relative to meteorites and the core? **Moynier *et al.*** (p. 1417, published online 24 February; see the Perspective by **McDonough**) found that the isotopic signature of chromium in a number of meteorites deviates from the bulk silicate portion of Earth. This effect suggests that chromium depletion from the mantle and enrichment in the core occurred because chromium followed iron and other metals down to the core during the differentiation of Earth's interior. Further modeling suggests that Earth's chromium isotope signature was imparted from Moon-sized masses that differentiated before colliding together to form Earth.

Titan's April Showers

The equatorial regions of Saturn's largest moon, Titan, are predominantly arid with vast expanses of dunes. Dry fluvial channels have been observed in these regions, which have been proposed to represent remnants of a past wetter equatorial climate. Using the Cassini Imaging Science Subsystem, **Turtle *et al.*** (p. 1414; see the Perspective by **Tokano**) observed sudden surface changes near Titan's equator after a cloud outburst. The best explanation for these changes is rainfall from a low-latitude storm in Titan's early northern spring, suggesting that the equatorial climate is not static year-round and that the fluvial channels may not necessarily be remnants of an early wetter climate.



Persistent Vibrations

In the gas phase, bimolecular reactions proceed in a tightly regimented fashion. Quantum mechanics dictates that only specific vibrations and rotations can accompany the electronic rearrangements associated with the scission and formation of bonds. In principle, these rules still apply in solution, but frequent collisions with surrounding solvent molecules tend to blur the energy distribution. By applying sensitive ultrafast spectroscopy, **Greaves *et al.*** (p. 1423, published online 3 February; see the Perspective by **Bradforth**) have now found that when CN snatches a hydrogen atom from cyclohexane in solution, the HCN product exhibits specific H-C stretching and bending motions that are only partially suppressed by solvent during the tens of picoseconds following its formation.

A Boost for Hydrogen Power

Hydrogen is in many respects an excellent automotive fuel: it is lightweight, releases a great deal of energy in reacting with oxygen, and forms a completely innocuous product—water—in the process. Unfortunately, the gaseous element is not so easy to store at high density. Hydrogen-rich solid compounds such as ammonia borane can be coaxed into releasing H₂ under fairly mild conditions, but the polymeric material left behind is difficult to replenish. **Sutton *et al.*** (p. 1426) now show that treatment of such material with hydrazine in liquid ammonia regenerates ammonia borane in high

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yield over the course of a day. Though only demonstrated at a small scale, the method bodes well for further development of ammonia borane as a hydrogen storage medium.

Selectivity Switch

Chiral molecular catalysts can usually be synthesized in either of their two mirror image forms (enantiomers) enabling preparation of either product enantiomer in the reaction they accelerate. However, to synthesize the independent catalysts often involves complex, multistep procedures.

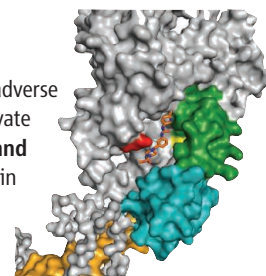
Wang and Feringa (p. 1429, published online 10 February; see the Perspective by **Ooi**) designed a single catalyst that, through photoisomerization, could be modulated between conformations that select for opposite product enantiomers in an organic reaction. The catalyst cycled through isomers with distinct helicities and a varied relative disposition of two functional groups (an amine and a thiourea) that needed to cooperate to activate the reagents.

Tortoise or Hare Evolution

The “mutator” phenomenon allows clones of asexually reproducing bacteria to evolve at different rates, for example, enabling bacteria to escape selection pressure by antibiotics. **Woods *et al.*** (p. 1433) compared *Escherichia coli* that had been collected and frozen at different points in a long-term evolution experiment. Surprisingly, after several hundred generations, clones that had initially showed low competitive fitness outcompeted initially more successful clones. The apparent losers turned into winners, not by increasing mutation rate and running off with the innovations, but because their competitors acquired mutations that, while conferring superior fitness in the short term, in the longer term hindered their “evolvability.”

Help for Heart Failure?

Heart failure is often caused by decreased cardiac contraction. Current therapies increase contractility indirectly by acting on signaling pathways, which can have adverse side effects. A small molecule, omecamtiv mecarbil (OM), has been shown to activate cardiac myosin directly. **Malik *et al.*** (p. 1439; see the Perspective by **Leinwand and Moss**) now show that OM acts allosterically to increase the transition rate of myosin into the strongly actin-bound force-producing state. OM increased cardiac function in animal models, which provides a proof-of-principle that direct cardiac myosin activation is a potential strategy for treating systolic heart failure.



Storage and Retrieval

In primates, visual long-term memory is stored in the inferotemporal cortex. Neuronal activity related to presented and retrieved images has been demonstrated at the single-neuron level. However, the underlying neuronal network dynamic remains largely unknown. **Takeuchi *et al.*** (p. 1443) investigated the functional interactions between different layers of the inferotemporal cortex while monkeys performed a paired associate memory task. While the cue stimulus was acquired, the direction of interactions followed the classical interlaminar pathway from granular via supragranular to infragranular layers as suggested by the anatomy of the canonical microcircuitry. During the subsequent delay period, when networks had to hold or retrieve the stimulus, the functional interactions indicated a reversal with infragranular neurons spiking before supragranular neurons.

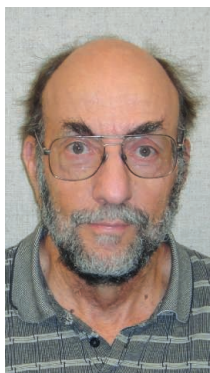
You're Not Alone

One of the key transitions for young people, especially in the developed world, occurs when they begin tertiary education and, in many cases, move away from home and out of their accustomed social milieu. Their capacity to adjust and to develop a new social network can influence their level of academic achievement, and these adjustments may be more challenging for some than others. **Walton and Cohen** (p. 1447) examined the effectiveness of supplying African Americans a 1-hour exercise designed to bolster their psychological well-being against events that might make them feel that they do not fit in or belong in higher-education environments. The intervention was associated with a 50% reduction of the gap in grades between African Americans and European Americans when assessed at the end of college, and appeared also to enhance physiological measures of well-being.

CREDIT: MALIK ET AL.



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Inspirational Chemistry

THROUGHOUT 2011, NATIONS ARE CELEBRATING THE INTERNATIONAL YEAR OF CHEMISTRY. This worldwide recognition of the importance of chemistry is somewhat unusual. It is true that chemistry has been called “the central science,” not only by chemists but even in Wikipedia (of course, that article may have been written by a chemist), perhaps as a metaphor for its role in connections between the fundamental concepts of physics and the practical problems of biology. Furthermore, it is the discipline that will continue to drive the discoveries that tackle today’s most vexing challenges: solving the energy problem, developing and producing new treatments for diseases, devising advanced materials for a host of applications, and many more. It seems most appropriate to talk of chemistry as overlapping, rather than bridging, other disciplines. And most certainly it is time to celebrate the creative future of chemistry, which lies in myriad directions.

So why does chemistry often take a back seat to other sciences? Traditionally (for most of the 20th century, at least), the paradigmatic face of science in the public view was physics; more recently, biology appears to be sharing (or even taking over) that role. Curiously, chemistry has never been able to claim a comparable place in the sun. Some people have argued that chemistry is ultimately reducible to physics; others, that chemistry is merely technology, a collection of enabling methodologies, posing no deep questions and offering no profound meaning. Although chemistry unquestionably has its own eloquent spokespersons, few if any are household names—counterparts to the likes of Stephen Hawking, Carl Sagan, or Stephen Jay Gould. The grand themes that attract so much interest to physics and biology—the origin of the cosmos, the workings of life, a theory of everything—are not so widely recognized in chemistry. Why is this so, inasmuch as almost everything is chemistry?

We suggest that the noble themes in chemistry are there, but may be a little harder to see. One can look outward to the universe, or inward to the mind, and recognize the complexity and profundity of the questions to be answered. The problems that contemporary chemistry tackles are just as fundamental, but may not be as immediately obvious to the non-chemist. We could illustrate this claim in many ways, but perhaps one has received the broadest sustained attention: photosynthesis. How can light be harvested and converted to electrochemical energy that is sent off so efficiently in two directions: to both reductively generate the building blocks of life from carbon dioxide and oxidize water to oxygen? This extraordinarily complex question, to be sure, is closely linked to aspects of both physics (but cannot be completely reduced to physics) and biology; but the answer clearly lies in the realm of chemistry. And the workings of each individual component, as well as the entire integrated system that nature has constructed, pose questions that are fully as deep and inspirational as those in any other field of science. Moreover, on the practical side, the answers will be needed to devise methods for making comparably effective use of solar power, which at present appears to be the only resource of sufficient magnitude to cope with the world’s long-term energy needs.

With a solution to this problem, we could not only stop turning all the fossil fuels generated by billions of years of natural photosynthesis into carbon dioxide, but even begin to reverse the process. Let’s take this International Year of Chemistry as a call to focus on such grand visions. By turning up the power of fundamental research to address daunting challenges, this could well become the Century of Chemistry.

— Harry Gray and Jay Labinger



BIOCHEMISTRY

Targeting Tryptophan

S-adenosylmethionine (SAM) seems like an innocuous combination of an amino acid and a nucleoside, yet it supports a wide variety of biochemical transformations whose scope remains underappreciated. Zhang *et al.* explore its intriguing role in the biosynthesis pathway of the antibiotic nosiheptide—a macrocyclic thiopeptide with an embedded indole. The first step is reductive cleavage of SAM to yield the 5'-deoxyadenosyl radical (5'-dA*) along with methionine. Surprisingly, 5'-dA* goes on in this case to abstract a hydrogen atom, not from the usual suspect (a carbon atom of the substrate), but from the indole nitrogen of tryptophan. This leads to a rather complicated sequence of events, with the final outcome being the release of the nitrogen atom and α -carbon (from the backbone portion of tryptophan) as ammonia and formaldehyde and the attachment of the carboxylate to the indole ring at C2; the indolic acid is then used to make nosiheptide. Having established this mechanism, the authors go on to introduce a fluorinated tryptophan derivative into the medium and show that the fluorinated indole functionality is incorporated (albeit somewhat less efficiently) into the corresponding final product, thereby broadening the structural diversity of this antibiotic class. — GJC

Nat. Chem. Biol. **7**, 154 (2011).

DEVELOPMENT

Hox9 Sets the Stage

An intricate interplay of signaling pathways, which include polarized gradients of Hand2 and Gli3 expression and subsequent Sonic hedgehog signaling, is important early on in anterior-posterior patterning in limb development. How these signaling gradients are established, however, is unknown. Xu and Wellik use mouse genetics to examine whether Hox9 genes, which are expressed along the forelimb anterior-posterior axis, play a role in early mouse limb development. No obvious defects are seen in mice with single

mutations of the Hox9 genes (Hoxa9, Hoxb9, Hoxc9, and Hoxd9); however, mutation of all four

Hox9 genes resulted in severe forelimb defects, with total loss of the posterior skeletal elements. This phenotype resembles the forelimb phenotype of Shh and Hand2 mutants. Subsequent analysis of in situ mRNA expression revealed that the



ECOLOGY

See You Next Summer

Socially complex species such as humans, elephants, wolves, and orcas establish strong bonds among individuals that persist after periods of separation. These "fission-fusion" dynamics require an ability to recognize individuals even after periods of absence. Such long-term individual recognition has often been attributed to large-brained species with strong socio-cognitive abilities; however, bats also display this ability. Whether these dynamics involve individual recognition or are purely driven by behavioral aggregation, however, is unknown. Bechstein's bats (*Myotis bechsteinii*) form stable colonies of females from April through September, which disintegrate during winter. Kerth *et al.* followed the movements of individually marked Bechstein's bats in two well-studied populations over 5 years. Analysis of 20,500 roosting sites revealed that individuals show consistent preference for individual roosting partners, who may differ in age, morphology, and family, and that these preferences persist over years, even after separation. These communities resemble those found in other socially complex species, which suggests that although social complexity is demanding sociocognitively, it may not require a large brain. — SNV

Proc. R. Soc. London Ser. B **278**, 10.1098/rspb.2010.2718 (2011).



Hox9 genes act upstream of Hand2 to initiate the posterior limb compartment and Hand2 polarity, which in turn initiates Shh signaling. No defects in hindlimb development were observed in Hox9 mutant mice, which suggested that the role of Hox9 genes in setting up limb polarity is restricted to forelimb development only. — BAP

Proc. Natl. Acad. Sci. U.S.A. **108**, 10.1073/pnas.1018161108 (2011).

CHEMISTRY

Ring of Rings

The abstract prospect of using a circle to derive a square has intrigued scholars of geometry for centuries. Segawa *et al.* have now used a square

to derive a circle. The arena in this case was chemistry, rather than mathematics, and the task at hand was to assemble a molecular ring composed in turn of 12 benzene rings, linked to one another at the diametrically opposed 1 and 4 positions. Previous efforts had yielded small quantities of this [12]cycloparaphenylene in a mixture of variously sized relatives bearing 9 through 18 benzenes. The key to a more efficient route was a building block linking two benzene rings at an approximate right angle through an intervening cyclohexane diol derivative. By thoroughly optimizing a catalytic nickel system, the authors succeeded in linking four such building blocks in a single reaction medium to form a square with the cyclohexane

rings at the corners and two benzenes along each edge. Dehydration/aromatization of the corner rings then honed the square into the circular targeted product in sufficient yield to afford a crystal structure. — JSY

Angew. Chem. Int. Ed. **50**, 10.1002/anie.201007232 (2011).

MICROBIOLOGY

Cystic Fibrosis Double Whammy

People with cystic fibrosis (CF) suffer from life-threatening antibiotic-resistant *Pseudomonas aeruginosa* respiratory infections, with consequent chronic inflammation, which generates oxidative stress. *P. aeruginosa* expresses several multidrug efflux systems, including the MexXY-OprM pump, which drives antimicrobial resistance in CF lungs. Expression of MexXY-OprM is unexpected, because ribosome disruption, but not antibiotics such as aminoglycosides, induces its expression, yet CF isolates exhibit high degrees of resistance to aminoglycosides. It turns out that *mexXY* up-regulation depends on the gene PA5471, which is induced by oxidative stress. Fraud and Poole now demonstrate that exposure to inflammation-induced oxidative stress for several days produced a fourfold elevation in aminoglycoside resistance in *P. aeruginosa*, which was indeed mediated by PA5471. Aminoglycoside resistance did not always follow increased *mexXY* expression alone, which suggests that additional genes required for translation or protein synthesis may be involved. Thus, chronic inflammation, rather than antibiotics, drives the expression of MexXY-OprM, which leads to drug resistance in CF lungs. — CA

Antimicrob. Agents Chemother. **55**, 1068 (2011).



studies revealed a narrow emission transition, suggesting single-chromophore behavior, but the emission was not completely polarized, implying that bending defects were present. The authors isolated the molecules at low concentration and obtained spectra at 4 K, and under these conditions, the spectra of the oligomer and polymer were virtually the same—a single 0-0 transition peak at 445 nm, with mirror symmetry between excitation and emission and some excitation of vibrational modes. Thus, only a single exciton is created in the polymer, and it can travel more than 50 times its length along the chain. — PDS

J. Am. Chem. Soc. **133**, 10.1021/ja109342t (2011).

CLIMATE SCIENCE

More and More Melting

One of the most important consequences of global warming is the loss of mass from the Antarctic and Greenland ice sheets, and the rise in sea level accompanying that loss. Measuring changes in the mass of an ice sheet is extremely difficult, due both to the technical challenges involved in collecting good data and the intrinsic variability of the process, but the precision of the applied methods has in-

creased sufficiently over the past decades that even monthly trends can now be discerned. Rignot *et al.* provide an update on the state of mass change of the Greenland and Antarctic ice sheets, using two independent methods in order to demonstrate consistency in the measurements. They find that the rate of ice sheet loss has accelerated over the past 18 years by a combined total of 36.3 ± 2 Gt/year², three times faster than that of mountain glaciers and ice caps. If this trend continues, ice sheets will be the largest single contributor to sea-level rise by the end of the 21st century and will probably contribute more to sea-level rise than is currently projected. — HJS

Geophys. Res. Lett. **38**, L05503 (2011).

CHEMISTRY

Practically Perfect Pi Chains

In principle, a planar π -bond network in a conjugated polymer chain should provide a perfect highway for delocalized charge carriers created by photoexcitation. In practice, what should be a rigid molecule has bends and defects that usually cause the polymer chain to segment into localized chromophores. Da Como *et al.* performed broad-band single-molecule photoluminescence (PL) and PL excitation (PLE) spectroscopy on β -phase poly(9,9-dioctylfluorene) oligomers (nine repeat units) and polymers (about 500 repeat units), in which all of the repeat units lie in one plane. Previous

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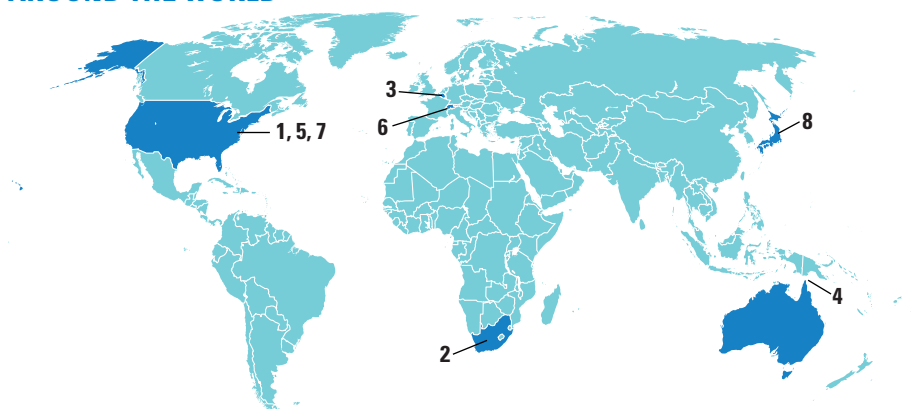
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AROUND THE WORLD



Silver Spring, Maryland 1

First Lupus Drug Approved In 50 years

The approval was as symbolic as it was scientific. Last week, for the first time since 1955, the U.S. Food and Drug Administration (FDA) gave the green light to a treatment for lupus. The therapy, called belimumab and marketed as Benlysta, is one of the first to be developed based on human genome research. It is far from perfect: FDA estimated that about 11 people need to get the monoclonal antibody for them to benefit. (The company's estimate was more favorable.)

Human Genome Sciences developed belimumab after it discovered a new protein in 1996 called BLyS, which helps regulate B cells. When BLyS levels rise in lupus patients, the disease often worsens.

More than two dozen lupus patients and advocates testified at a November FDA advisory meeting to discuss the therapy, pleading for approval. "Benlysta may not be the magic to help Aiden," said one woman, whose 17-year-old daughter has severe lupus, "but by approving it, you will be giving hope to those affected with lupus and their families."

South Africa 2

Old Satellite Dishes Look to the Stars

When radio astronomers map the world's arrays of dishes that work together to survey the skies, Africa is almost blank. Now astronomers in South Africa hope to fill that gap by converting old telecommunications dishes, which are being dismantled as fiber-optic cables are laid, into radio telescopes to produce a low-cost, continent-spanning array.

The group, which includes scientists at South Africa's Square Kilometre Array



(SKA) project office, whose telescopes are shown here, thinks that about 20 viable dishes are sprinkled over the continent. Once commandeered, the dishes will need new detectors and probably adjustments to their steering motors. South African astronomers are already competing with Australia to host the potentially gap-filling SKA, which will comprise thousands of receivers spread over thousands of kilometers. "We thought, why wait for SKA? Why not see what we can do now?" says Justin Jonas, an associate director in the project office.

<http://scim.ag/old-dishes>

Brussels, Belgium 3

E.U. Patents for Embryonic Stem Cell Tech in Doubt

Inventions involving cells that are derived from human embryonic stem (hES) cells are not patentable, according to a preliminary opinion by the European Court of Justice. In the 10 March opinion, Advocate General M. Yves Bot said although hES cells are not embryos, because they are derived from human embryos, patents involving them are not allowed under E.U. law.

The case involves a German patent granted to neuroscientist Oliver Brüstle in 1999 covering a method for turning mammalian ES cells into neurons. In 2004, Greenpeace challenged the patent on the

grounds that a 1998 E.U. directive on biotechnology patents prohibits patents on "uses of human embryos for industrial or commercial purposes."

Bot's opinion is not binding on the court's judges, who are expected to issue their ruling in the coming months. One of Brüstle's attorneys, Clara Sattler de Sousa e Brito, notes that four E.U. member countries and the European Commission submitted legal opinions that found that inventions involving already-derived hES cells should be patentable. The full court "would have a hard time completely ignoring them," she says.

Torres Strait, Australia 4

British Museum Returns Human Remains to Australian Aborigines

The decorated skulls, teeth, and other remains of 119 ancient humans will be returned to their homeland in the Torres Strait Islands between Australia and Papua New Guinea, according to an agreement signed by the trustees of the Natural History Museum in London last week. The remains were originally traded to missionaries, then to officers and naturalists aboard Naval Survey ships in the early to mid-19th century. During 18 months of negotiations with Aboriginal residents of the Torres islands and the Australian government, museum scientists explained the importance of the remains, which, as part of the museum's huge collection of human specimens, have helped trace patterns of migration between ancient populations.

To ensure that the remains stay in good condition, and to keep the science going, the museum has offered to train an islander to handle and study the specimens in London before the move. The museum also reserved the right to retain the collection if it appears likely to be destroyed.

<http://scim.ag/torres-remains>

Washington, D.C. 5

Flap Over Shipwreck Exhibit

Archaeologists are criticizing the ethics of a planned Smithsonian Institution exhibit, *Shipwrecked: Tang Treasures and Monsoon Winds*, slated to open in the Arthur M. Sackler Gallery in 2012. The exhibit is based on artifacts salvaged in the late 1990s by a private company, Seabed Explorations GbR, from an Arab dhow that sank in the Java Sea in the 9th century C.E.

Critics say that the company did not

observe professional archaeological standards while recovering the artifacts, which include glazed ceramics (pictured), lead ingots, and intricately worked vessels of silver and gold from the Tang dynasty. Many archaeologists also say that the later sale of the objects to another company for a reported \$32 million contravenes their field's ethical guidelines.



In recent weeks, three major American archaeological associations and three of the Smithsonian's own research organizations have written to Smithsonian Institution Secretary Wayne Clough to oppose the exhibition. But Julian Raby, director of the Arthur M. Sackler Gallery, remains a firm supporter of the exhibit, noting that no laws were broken during the salvage operation. <http://scim.ag/tang-artifacts>

Geneva, Switzerland 6

WHO Gets Mixed Reviews For H1N1 Response

Eight months after the World Health Organization (WHO) declared the swine flu pandemic officially over, an independent expert group has given the global health agency a decidedly mixed evaluation of how it handled the episode. The criticism comes in a draft report by a panel of 25 outside experts convened by WHO to review both the organization's response to the pandemic and its new International Health Regulations (IHR), which mandate that countries cooperate more closely with the agency during health threats that cross borders.

The report, made public by WHO on 10 March, knocks WHO for causing confusion about the severity of the pandemic and for "numerous systemic difficulties" that slowed the production of the vaccine and delivery to poor countries.

The panel praised the IHR—which only went into effect in 2007 and had never before had a real-world test—and WHO for rapidly kicking into action a global surveillance network, helping countries track the virus and contain its spread. But it warned that had this virus caused more severe disease, "the unavoidable reality is that tens of millions of people would be at risk." <http://scim.ag/WHO-critique>

Washington, D.C. 7

Overhaul of U.S. Patent System Advances

The U.S. Congress is moving toward a new patent standard that most of the world uses already. Senators Patrick Leahy (D-VT) and Orrin Hatch (R-UT) last week persuaded the Senate to vote overwhelmingly (95–5) for their Patent Reform Bill (S. 23), which would award patents to the first person to file a valid application on an invention, instead of the person who claims to have conceived it first.

Advocates say the change would make the U.S. system more objective and predictable. The bill also would add a new procedure for challenging patents after they've been awarded and would create an independent fund backed by fees to pay for the work of the U.S. Patent and Trademark Office, with the goal of insulating the agency's budget from congressional politics. (Partly because of funding problems, the patent office has a backlog of more than 700,000

THEY SAID IT

"You have only got to look at the distress caused in these families to see the need for this."

—Douglass Turnbull of Newcastle University, welcoming last week's launch of a U.K. review of an IVF technique developed by his team. The technique would prevent severe inherited diseases by transferring parental DNA from a fertilized egg with defective mitochondria to another woman's healthy egg, resulting in an embryo with DNA from three different people.

applications.) The action now moves to the House of Representatives, where Judiciary Committee Chair Representative Lamar Smith (R-TX), who favors the first-to-file standard, says a reform bill will be introduced "this month."



Fukushima Prefecture, Japan 8

Earthquake-Shattered Japan Confronts Nuclear Crisis

As Japan reeled from the devastation wrought by last week's magnitude-9.0 earthquake and subsequent tsunami, the country faced another potential disaster: a nuclear fuel meltdown at the Fukushima Daiichi Nuclear Power plant 220 kilometers north of Tokyo. After generators powering the cooling systems were knocked out, probably by the tsunami, hydrogen explosions on 12 and 14 March destroyed the outer containment buildings of two reactors, while a third reactor lost coolant. This satellite photograph shows the stricken reactor complex shortly after the second explosion. On 15 March, as *Science* went to press, an explosion and fire in an area where highly radioactive used fuel rods are kept cool heightened fears of the massive release of radioactivity. Japanese media have reported that the Fukushima plants were built to withstand an earthquake of magnitude 8.2. For more on the earthquake, see p. 1375 and http://scim.ag/japan_quake2011.

NEWSMAKERS

Nuclear Watchdog to Run For Egypt's Presidency

Mohamed ElBaradei, former chief of the United Nations nuclear watchdog agency, has announced his bid for the presidency of Egypt. Speaking on Egyptian television last week, ElBaradei said, "When the door of presidential nominations opens, I intend to nominate myself." He emphasized that he would run only if truly democratic elections were held, and urged that a scheduled 19 March referendum on constitutional amendments, set by military rulers, be postponed or canceled in favor of drafting a new constitution.

ElBaradei emerged as a central figure among opposition leaders during the protests that led to the resignation of President Hosni Mubarak (*Science*, 11 February, p. 659). A lawyer by training, ElBaradei shared the 2005 Nobel Peace Prize with the International Atomic Energy Agency, for which he served as director general from 1997 to 2009, for contributions to nuclear security.



New JAMA Editor-in-Chief

A Boston pediatrician, Howard Bauchner, will be the next editor-in-chief of the prestigious *Journal of the American Medical Association (JAMA)*, taking the reins this



summer from Catherine DeAngelis. DeAngelis, who may be best known for tackling conflicts of interest in scientific publishing, announced last fall that she would step down after 11 years.

Bauchner has spent the bulk of his career at the Boston University School of Medicine, and his research has focused on improving the quality of health research and clinical trials. He has published on everything from discordance in how children are screened for developmental delays to how to prevent prescribing errors in a pediatric emergency department. Bauchner is currently the editor-in-chief of *Archives of Disease in Childhood*. In a statement, he said he wants "to make sure *JAMA* contributes to the discussions in American medicine," including health care reform and genetics.

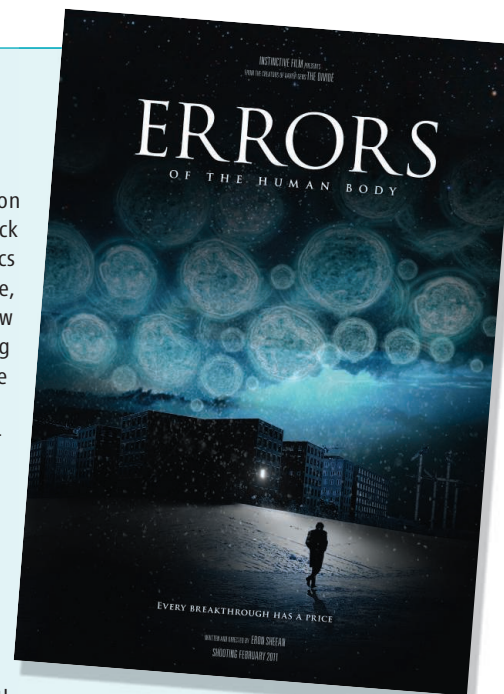
Random Sample

From Cells to Celluloid

Five years ago, Australian screenwriter Eron Sheean spent several months at the Max Planck Institute of Molecular Cell Biology and Genetics in Dresden, Germany, as an artist in residence, "getting a sense of the place," he says. Now he's back with a film crew and actors (including *Perfume's* Karoline Herfurth) to shoot a feature film there.

Errors of the Human Body "is a little science fiction and a little science fact," says Sheean. In the film, a Canadian geneticist named Geoffrey Burton moves to a Dresden lab determined to cure the rare disorder that took his child's life. Research is Burton's way of "solving his emotional problem," Sheean says, and it eventually makes him paranoid. "I see it as a character drama with elements of a thriller," he says. A "mood teaser" on the film's Web site (<http://scim.ag/errors-film>) shows scientists peering through microscopes in darkened labs; fluorescent lights blink on as a figure stalks the institute at night.

Sheean hopes to debut the film at the Toronto International Film Festival in September. Shooting at the lab has gone smoothly, says producer Darryn Welch. "We're cheating in a few areas—we don't have access to the BSL-4 [biosafety level 4] rooms—but other than that we have access to the whole place." One change: replacing the lab's mice with axolotls, eerie-looking white salamanders known for their ability to regenerate lost limbs. As for how the institute's scientists—and the film's de facto extras—have adjusted to life on set, says Welch, "I'm sure they've seen stranger things through a microscope."



FINDINGS

Getting to the Guts of Malnutrition

VANCOUVER, CANADA—A roomful of mice in St. Louis, Missouri, may offer a surprising explanation for why some starving children are susceptible to malnutrition while others fare better: variations in gut bacteria.

Microbiologist Michelle Smith of Washington University in St. Louis fed germ-free mice stool samples from a set of twins in Malawi, one of whom, despite eating the same food as the other twin, suffers from a form of malnutrition called kwashiorkor in which



a child's belly and face become bloated. Once the children's gut bacteria had taken hold in the rodents' intestines, Smith and her colleagues manipulated their diets, checking for changes in the gut bacteria and weighing the rodents every few days.

Boosting and then cutting back their diet caused mice with gut bacteria from the child with kwashiorkor to lose and then gain more weight than mice with bacteria from the healthier twin, Smith reported at the International Human Microbiome Congress here. The composition of the gut flora, and the makeup and abundance of bacterial enzymes, also fluctuated far more in the mice with bacteria from the child with kwashiorkor. <http://scim.ag/gut-nutrition>

Microbes Give Mice Intestinal Fortitude

VANCOUVER, CANADA—Infect some strains of lab mice with the pathogen *Citrobacter rodentium* and they die, yet other strains survive. You might think the difference is due to

CREDITS (TOP TO BOTTOM): INSTINCTIVE FILM; MOHAMED OMAR/EPA/LANDOV; JAMA; LYLE CONRAD/CDC

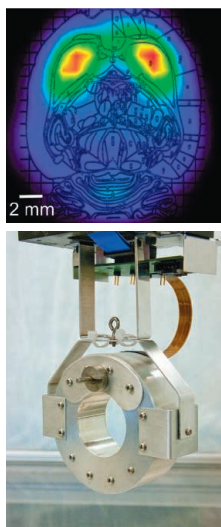
genetics, but it's not: The survivors can thank their gut bacteria.

Microbiologist B. Brett Finlay of the University of British Columbia, Vancouver, and colleagues used a high dose of antibiotics to kill all the native gut bacteria in a susceptible strain of mice and then fed those mice fecal material from a resistant strain. Microbes in the fecal material took up residence in a susceptible mouse's gut for about a month. When infected with *Citrobacter*, the once vulnerable rodents lived to tell the tale, Finlay reported at the International Human Microbiome Congress here last week.

Finlay and colleagues then turned the tables. They gave resistant mice low doses of an antibiotic that altered the relative abundance of various gut microbes but didn't kill them altogether. *Citrobacter* caused severe diarrhea in these rodents. The researchers found that the shift in the microbial makeup reduced by half the thickness of the mucous layer that typically lines the gut. "By thinning this mucous [layer], we increased the susceptibility to infection," Finlay said at the meeting. <http://scim.ag/gut-resist>

High-Tech Hat for Brain-Scanning Rats

Rats don't like having their brains scanned any more than humans do, so studying their brains with positron emission tomography (PET) usually requires that they be asleep. Now researchers at Brookhaven National Laboratory in New York state have devel-



The RatCAP imager (bottom) produced this PET scan (top) from a moving rat.

without using anesthesia, which alters brain chemistry and could misdirect experiments.

The RatCAP imager, equipped with miniature solid-state photosensor arrays, sits on a rat's head and is attached to a readout system that encircles the animal's head like a very high-tech flea collar. A flexible arm relieves the weight of the scanner and allows the rats to run about their cage, move their heads up and down, and turn 360°.

When the researchers injected rats with a mildly radioactive tracer to measure dopamine levels in the brain—first in a conventional PET scanner under anesthesia, then when wearing a RatCAP—they found that the patterns of dopamine activity differed, suggesting that anesthesia could be having more of an effect on brain metabolism than

oped a device called a Rat Conscious Animal PET (RatCAP) imager, which allows a rat to wear a scanner even while it moves around. The imager, described online in *Nature Methods* this week, will allow behavioral neuroscientists to study metabolic activity in the brain

BY THE NUMBERS

80 million Hectares of surface freshwater in Canada's boreal forest, the largest supply in the world, according to a report by the Pew Environment Group, which is calling for 50% of the forest to be protected.

€153 billion Estimated contribution of insect pollinators to global food production, according to a United Nations report examining threats to honeybees in different regions.

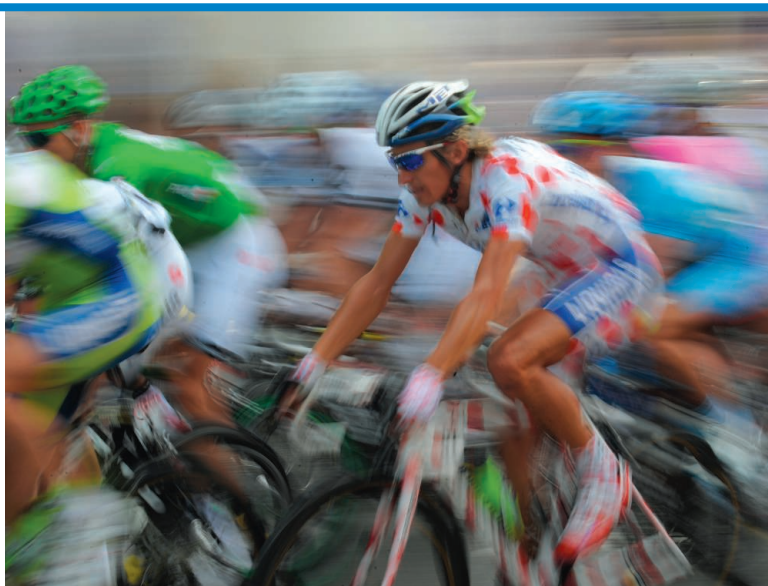
10 Number of last year's "hot papers" that were penned by genomicist Eric Lander, the most by any researcher, according to Thomson Reuters. About 0.1% of scientific papers are deemed "hot," meaning they garner an exceptional slew of citations soon after publication.

previously thought. The researchers are in the process of commercializing the technology so that others can use it to study the neurobiology of disorders such as depression, anxiety, and seizures.

<http://scim.ag/RatCAP>

New Doping Test Means Cyclists Can't Pull a Fast One

A new technique for detecting blood doping in athletes has received a vote of confidence in the sports world. The Court of Arbitration for Sport—considered the world's authority in sports disputes—last week upheld the decision by the International Cycling Union (UCI) to bar Italian cyclists Franco Pellizotti (pictured) and Pietro Caucchioli from competing for 2 years. The two cyclists tested positive for doping using UCI's new Athlete Biological Passport (ABP), approved by the World Anti-Doping Agency in 2009. Developed by researchers at the Swiss Laboratory for Doping Analyses in Lausanne, ABP ties an athlete's sex, age, ethnicity, and exposure to altitude to several blood measurements, such as levels of oxygen-binding hemoglobin, which athletes sometimes increase by illegal blood transfusions or hormone injections. Using previous measurements to establish a personalized baseline, the test flags athletes who have abnormal readings as suspected doping cases.



CREDITS (TOP TO BOTTOM): BROOKHAVEN NATIONAL LABORATORY; PATRICK HERTZOG/AFP PHOTO/NEWS.COM



Utter destruction. Noda village in Iwate Prefecture was wiped out by the tsunami.

JAPAN DISASTER

Devastating Earthquake Defied Expectations

TOKYO—Even for a nation inured to temblors and bracing for the Big One, last week's devastating earthquake and tsunami were beyond imagination. Experts, too, were caught off-guard. "I never thought this kind of [event] could happen" in this region, says Hiroo Kanamori, a seismologist at the California Institute of Technology in Pasadena.

The earthquake's astonishing power and unexpected location also expose the futility of forecasting where and when the next Big One will hit, says Robert Geller, a geophysicist at the University of Tokyo. Even in a country as extensively instrumented and thoroughly studied as Japan, he says, major quakes always "seem to be ones not expected."

The 11 March Tohoku earthquake ranks among the five strongest temblors recorded by modern instrumentation. The U.S. Geological Survey and the Japan Meteorological Agency now peg the magnitude at 9.0. The quake ruptured more than 400 kilometers of crust along the Japan Trench subduction zone, where a tectonic plate is diving beneath the northeast coast of Honshu Island. Its epicenter was 130 kilometers east of Sendai, a city of about 1 million people in Miyagi Prefecture, and 373 kilometers northeast of Tokyo. Authorities expect the death toll to top 15,000. Tsunami waves that exceeded 7 meters in height when they came ashore washed away scores of communities and inflicted damage as far away as Califor-

nia. The tragedy may yet be compounded by ongoing crises at two nuclear power plants, where workers were racing to prevent reactor core meltdowns and contain radiation leaks as *Science* went to press.

Japan sits on the Pacific Ring of Fire, where about 90% of the world's earthquakes occur as tectonic plates bump and grind over and under one another. Along the Japan Trench, the Pacific Plate is being forced under the Okhotsk Plate. In some regions, the subducting plate slides smoothly under the overriding plate. In other areas, plates couple,

or stick together, at their interface. If strongly coupled, the plates' boundary regions are twisted until the rock can no longer bear the strain. At that point, a slip or rupture along the boundary allows the plates to snap back into lower-stress positions, releasing accumulated energy as earthquake waves. The concurrent sea-floor movement produces tsunamis.

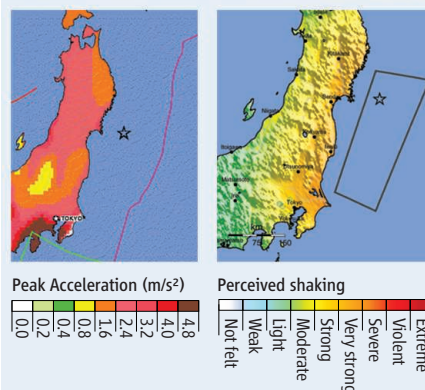
To assess earthquake risk in a given region, scientists look to past events for guidance. Records for northeastern Japan go back more than a century. Along the Japan Trench, earthquakes of magnitude 7 to about 8.3 have struck at 30- to 50-year intervals. According to Kanamori, recent earthquakes along the subduction zone relieved so much stress that it would have been hard to see where in the region enough strain had accumulated to produce a great earthquake. "Truthfully, we didn't foresee this," says Takuya Nishimura, a geodesist at the Geospatial Information Authority of Japan in Tsukuba who co-authored a 2004 paper in *Geophysical Journal International* detailing the buildup of crustal deformation in the region.

An earthquake was anticipated off the coast of Miyagi where the 11 March event occurred, but it was expected to be much smaller. Historical records and instrumental observations indicated that for this smaller section of the subduction zone, earthquakes of magnitude 7.5 or so recur every 30 to 40 years. The most recent was a magnitude-7.4 event in 1978 that killed 28 and triggered a small tsunami. The rupture zone of the 11 March earthquake "overlaps but is much greater than the source area of the 1978 earthquake," which ruptured only about 50 kilometers of the fault, Nishimura says.

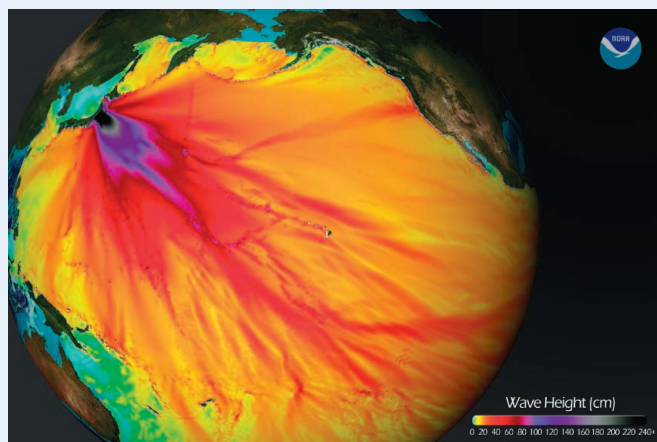
Peering deeper into the past, researchers a few years ago recognized an event of similar intensity to the 11 March disaster. Historical records tell of a large earthquake and the coastal plain becoming a "wilderness of water" in the year 869. A telltale marine sand layer buried in marshy deposits on the Sendai Plain revealed that the ancient tsunami must have run as much as 3 to 4 kilometers inland from the coastline at the time. Based on the extent of the inundation, researchers estimated the magnitude of the Jogan earthquake at roughly 8.3. Local media report having found traces of last week's tsunami 5 kilometers or more inland.

If the Tohoku earthquake was a reprise of Jogan, supercycles of massive earthquakes

RISKS AND REALITY



Unexpected location. The 11 March quake shook up a region (right) outside the areas expected to bear the highest seismic risks (left).



Waves of Destruction

Minutes after a powerful earthquake struck off the coast of Japan at 2:46 p.m. local time on 11 March, the Japan Meteorological Agency (JMA) issued tsunami warnings that were broadcast on TV and radio. Augmenting the warnings were local sirens and announcements. Still, most deaths and much destruction were the handiwork of the tsunami: Many victims knew the waves were coming but could not escape or didn't try until it was too late.

Residents of Japan's northeast coast were attuned to the risk. Tsunamis have periodically pounded the region, much of it a low-lying plain. An earthquake in Chile in 1960 triggered a tsunami that crossed the Pacific and killed 122 people in Japan. After that quake, 10-meter-high tsunami walls were built at locations such as harbor entrances throughout northeast Japan, says tsunami geologist Kazuhisa Goto of Chiba University in Japan. Elsewhere the coast is lined by berms, intended primarily to protect against storm surges.

These berms proved no match for the towering tsunami waves last

Code red. Computer-generated prediction of wave heights as the tsunami sped across the Pacific Ocean on 11 March.

week. Video clips aired by Japan's national TV station, NHK, indicate that many residents heeded warnings or common sense and headed for higher ground. But "the Sendai plain is quite flat, so it is difficult to escape," Goto says. JMA instruments detected the towering tsunami arriving in some areas just 30 minutes after the quake, giving residents little time to flee.

Some multistory concrete buildings in the area—refuges of last resort—were not tall enough. Local media found a survivor who had joined others on the roof of a three-story building in the coastal town of Minami Sanriku. According to the man, waves washed over the roof, some 13 meters high, and swept away everybody else. He survived by climbing a ladder attached to a roof-mounted antenna.

Stringent building codes are credited with having limited structural damage from the intense shaking of last week's earthquake, which was 500 times bigger than the one that flattened buildings in Christchurch, New Zealand, last month. But there are few options for girding against monster tsunamis. Planting trees or other vegetation along coasts can help blunt the force of the waves, but buildings must be located far inland. Planners have floated measures such as building more extensive sea walls, erecting stout towers as vertical refuges, and perching buildings on pillars to let water flow underneath. Such "provisions are quite expensive and difficult," Goto says.

The most effective tsunami-mitigation strategy may be to ensure that there are adequate escape routes—and educate people to run for their lives when the sirens blare. In the Sendai area, Goto says, elders who lived through earlier tsunamis were well aware of the danger. "But younger people do not know about those events," he says. Heart-rending live TV coverage showed that many people did not attempt to reach higher ground until the waves were upon them. "This will be a case study," says Joanne Bourgeois, a University of Washington, Seattle, tsunami geologist currently at Hokkaido University in Sapporo. Japan "has planned for these kinds of events. We can look at what worked and what didn't work."

—D.N.

may rock the region on 1000-year time scales, Nishimura says. Supercycles, of course, would not be limited to one segment of the Japan Trench, he says: "This event suggests that such kinds of great earthquakes might occur in other subduction zones." One candidate is the Cascadia fault, which runs off the coast from northern California to southern British Columbia. Last week's earthquake "is going to be the benchmark for the Pacific Northwest when the Cascadia fault breaks," says seismologist John Vidale of the University of Washington, Seattle. "We know that it can have an earthquake of this magnitude. It's a question of when, not if."

One worry is how the 11 March earthquake may influence neighboring sections of the Japan Trench. "We are really concerned" that the release of stress offshore of northeast Honshu has increased the chances of a large quake much closer to Tokyo, says seismologist Shinji Toda of Kyoto University. His preliminary calculations suggest that the earthquake loaded stress onto the fault segment

offshore of Boso Peninsula, a finger of land separating Tokyo Bay from the Pacific Ocean. Historical records indicate a large earthquake and tsunami battered the area in 1677.

The Tohoku earthquake will offer sobering lessons for risk assessment. Since 1965, Japanese seismologists have diligently watched real-time data from hundreds of instruments in the Tokai region west of Tokyo. If a fault along the Nankai Trough were

Online sciencemag.org

S Podcast interview
with author Dennis
Normile.

to grow restless, a panel of seismologists would assemble on a moment's notice to decide whether to warn of an impending magnitude-8 earthquake. Authorities have strengthened public buildings to withstand intense shaking and have built dikes and floodgates along the coast to thwart the anticipated tsunami from such a temblor.

But Japan's two great earthquakes of the past 2 decades—the 11 March event

and the Kobe earthquake of 1995—showed that experts were looking in the wrong place. Similarly, Kanamori says, in California attention is focused on the San Andreas fault, even though the most damaging earthquakes of the past several decades—including the 1994 magnitude-6.7 Northridge and the 1971 magnitude-6.6 San Fernando earthquakes—occurred elsewhere.

Researchers are able to estimate how much seismic strain will accumulate in a fault over time. "What we cannot predict is the individual sequence. It may be released in a single earthquake or may be released in smaller events," Kanamori says. "In view of the inevitable uncertainty, in my opinion it's better to have a more general approach [to earthquake preparation] than to have a prioritized, focused effort." For a nation that prides itself on preparedness, a distressing realization might be that some earthquakes are just too big, and too rare, to prepare for.

—DENNIS NORMILE

With reporting by Richard A. Kerr and Sara Reardon.

CREDIT: NOAA

ASTRONOMY

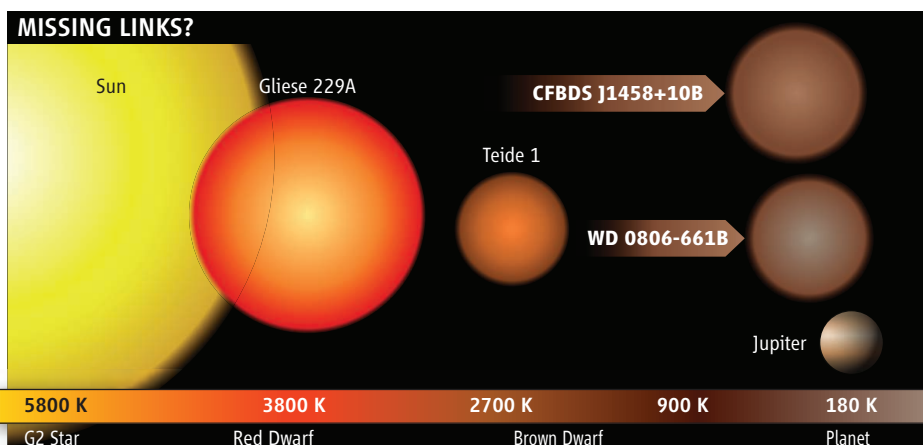
Cold Almost-Stars May Herald Hordes of Unseen Lurkers

Last year on Thanksgiving Day, astronomer Kevin Luhman was at his brother's house using his laptop to analyze images from the Spitzer Space Telescope when he spotted something that would make him profusely thankful. While family members were preparing to roast the turkey, Luhman discovered a pinprick of light that he suspected was a brown dwarf—a “failed star” too small to sustain the stable burning of hydrogen. “Based on how faint it was, I knew it had to be very cool and very low mass,” says Luhman, a researcher at Pennsylvania State University, University Park, who shared his excitement with his brother, a fellow astronomer who specializes in policy.

The object turned out to be the coldest brown dwarf ever seen, with a temperature of 300 kelvin, Luhman and his colleagues report in the 20 March *Astrophysical Journal Letters*. That's about as warm as a bright summer day on Earth. Meanwhile, other astronomers, led by Michael Liu of the University of Hawaii, Honolulu, have found another very cold brown dwarf whose estimated temperature is about 370 K, the researchers report in an upcoming paper in *The Astrophysical Journal*. Luhman's dwarf is 200 K cooler than the previous record-holder.

“These are very interesting discoveries,” says Mark Marley, a planetary scientist at the NASA Ames Research Center in Mountain View, California, who was not connected with either paper. Scientists knew in theory that such objects ought to exist, Marley and other astronomers say; now they have found them. The two objects could be the first examples of a proposed class of ultra-cool brown dwarfs known as the Y-class. And because the objects are almost as cold as “gas giant” planets—Jupiter is about 150 K—studying them could offer insights into planetary atmospheres.

Less than 8% as massive as our sun, brown dwarfs occupy a place between stars and planets. Since the discovery of the first brown dwarf in 1995—Gliese 229B, with a temperature of 950 K—astronomers have searched the skies for colder and colder brown dwarfs in the belief that such objects form a continuum between the puniest stars and the biggest planets. Their varied masses and temperatures give them a wide range of physical properties. So-called L-class dwarfs, whose temperatures range from 1500 K to 2000 K, have clouds of minerals and iron, whereas their proposed Y-class counterparts



Cool customers. Two newly discovered brown dwarfs—the chilliest ever discovered—may be members of a long-predicted but elusive class of objects straddling the boundary between stars and planets.

are expected to have clouds of water ice.

Many brown dwarfs have been found in binary systems with real stars. That's why the search for brown dwarfs often involves looking carefully in the vicinity of a known star in the hope of detecting a companion object lurking in the stellar glow. Luhman and his colleagues took that approach, using mid-infrared images from Spitzer to study the surroundings of some of the stars nearest to the sun. “We obtain two images of a star separated by a few years and check for any faint points of light that move across the sky in the same manner as the star,” Luhman explains. He says he looked at images of nearly 400 stars without any luck before spotting a companion to a white dwarf star known as WD 0806-661, some 63 light-years from Earth.

In addition to estimating the object's ultra-cool temperature, the group calculates that WD 0806-661B is about seven times as massive as Jupiter. Luhman and his colleagues hope to get spectra of the object from the Hubble Space Telescope, which could reveal whether it has water clouds in its atmosphere.

Last summer, Liu and his colleagues used the adaptive optics of the Keck telescope atop Mauna Kea in Hawaii to get a clearer image of what had previously looked like a single 600-K brown dwarf some 75 light-years away. The high-resolution scrutiny revealed two objects, one of which, named CFBDS J1458+10B, they identified as a brown dwarf with an estimated temperature of 370 K. Like the other team, Liu and his colleagues “hope to find water clouds” in the object's atmosphere.

If Hubble obtains spectra of the two brown dwarfs, that will be “exciting for several rea-

sons,” Marley says. “First, as giant planets age, they cool. So seeing these objects at 300 or 400 K is like looking at Jupiter in its youth. Secondly, there is lots of interesting physics that happens over this temperature range.”

Both teams think many more such chilly almost-stars are waiting out there, hard to find because they are so cold and faint. “We believe the bulk of the population of brown dwarfs are in this cold regime,” says Adam Burgasser, an astronomer at the University of California, San Diego, one of Luhman's co-authors. “The galaxy is about 10 billion years old, and brown dwarfs that have formed throughout the age of the galaxy have simply had a long time to cool off. So in order to really know how many brown dwarfs there are in the galaxy, and in the vicinity of the sun, we have to count up these very cold ones that are just being found for the first time.”

Davy Kirkpatrick, an astronomer at the California Institute of Technology in Pasadena who was not connected to the two finds, agrees. “These objects demonstrate the star-formation process was producing low-mass objects billions of years ago, but we don't yet know how frequently these objects were created or what their spectra look like,” he says.

Astronomers should be able to get a better handle on the prevalence of brown dwarfs once they've had a chance to analyze data brought in by the Wide-field Infrared Survey Explorer (WISE), a NASA space telescope whose goals include the search for brown dwarfs. WISE, which surveyed the skies for 14 months following its launch in December 2009, is scheduled to release its first set of data on 14 April.

—YUDHIJIT BHATTACHARJEE

Attack on Climate Studies Would Shutter Entire DOE Biology Program



Looking skyward. Dual-band scanning radar at the ARM site in north-central Oklahoma became operational this month.

a wait-and-see attitude toward the proposed decimation of BER. “We’re facing a lot of challenges in 2011” is as far as William Brinkman, head of the Office of Science, would go during a talk at BERAC’s regular meeting last week in Washington, D.C.

But a PowerPoint presentation by acting BER chief Sharlene Weatherwax hinted that things were anything but normal. Under the heading “Many proposals, much confusion, few answers,” her opening slide showed a roller-coaster car plunging down a slope. And Paul Gilna, who directs one of three 5-year, \$25-million-a-year bioenergy research centers that BER launched in 2007, doesn’t mince words when asked what would happen if the House bill were enacted. “For all intents and purposes, H.R. 1 closes down BER,” he says.

Low profile, high impact

As its name implies, BER has a twin focus on biological and environmental/climate science, and its budget is divided almost equally between the two. Both components make competitive awards to individual investigators. In addition, the biology program supports three bioenergy research centers—Gilna leads one based at Oak Ridge National Laboratory in Tennessee, and the others are at Lawrence Berkeley National Laboratory (LBNL) in California and the University of Wisconsin, Madison. Each has partnered with dozens of research teams around the country to develop new biofuels that would lessen the country’s dependence on foreign oil. BER also funds the Joint Genome Institute, a national user facility at LBNL for sequencing and understanding the functions of environmental and energy-related microbes and plants.

The environmental/climate program also operates large user facilities. One, the Environmental Molecular Science Laboratory at Pacific Northwest National Laboratory in Richland, Washington, is a world leader in using proteomics—changes in the entire complement of proteins expressed in cells—to explore thorny environmental and energy problems. The second, the Atmospheric Radiation Measurement (ARM) climate research facility, draws on a global network of ground and mobile stations to study clouds and other properties of the atmosphere, notably aerosols. Its unique capabilities enable scientists to shed light on these poorly understood but vital components of climate change.

Scientists were caught by surprise when they found out that the Department of Energy’s (DOE’s) office of Biological and Environmental Research (BER) would be shut down if a 2011 spending bill passed last month by the House of Representatives holds sway (*Science*, 25 February 2011, p. 997). The new Republican House majority has pledged to trim the federal deficit by cutting spending—and to be sure, House leaders are not fans of government-funded climate change research.

But researchers also say the assault on BER—one of several agencies that support climate research—reflects a fundamental misunderstanding of the office’s role in generating data for the global climate models that many Republicans love to hate. And climate science is only a small part of what BER does. The blunt attack on BER’s climate program would also cripple the office’s efforts in systems biology, genomics, bioenergy, and environmental remediation. Some programs trace their roots back to the dawn of the atomic age. Advocates hope that both BER’s pedigree and its broad portfolio of interdisciplinary basic research will earn it a reprieve when Congress strikes a final compromise on the 2011 budget.

“It’s a little bit like killing the messenger,” says Gary Stacey, chair of the office’s external advisory council (BERAC) and a professor of plant sciences at the University of Missouri,

Columbia. “BER doesn’t make [climate] policy. It funds scientists to provide the basic research that will improve the models, which the policymakers can then use or disregard.”

“I’m baffled by this proposal,” says Anna Palmisano, who headed BER until she retired from the federal government last November, noting that the office has always enjoyed good relations with Congress. “This isn’t some nibble. It’s a draconian step that reflects some deep-seated hostility to what BER does.”

The cuts are contained in a single sentence of a 380-page appropriations bill (H.R. 1) that proposes a \$61 billion reduction from current spending levels. In addition to shrinking by 18% DOE’s \$4.9 billion Office of Science, which oversees BER and five other programs, legislators singled out BER by capping its spending for the year at \$302 million. Given that the fiscal year is nearly half over, the cap represents a cut of almost 50% in an annual budget of \$588 million, effectively leaving BER with no money to operate.

Last week, the Senate rejected both the House bill and a gentler alternative offered by Senate Democrats that did not mention BER and makes a much smaller, untargeted cut to the Office of Science. That sets the stage for the next round of budget negotiations, with the House proposal a sword of Damocles for BER. In the face of that fiscal uncertainty, DOE officials have adopted

A Strong Defense of Science—and a Stiff Upper Lip

This too shall pass.

Last week, *Science* sat down with several members of the advisory council for the Department of Energy's Office of Biological and Environmental Research (BERAC) to discuss the massive cuts being proposed for the program (see main text). Although they were angered and saddened by the recent turn of events, none viewed the attack on BER as a rejection of the value of basic research by policymakers. "We clearly have to do something about the deficit," said Gary Stacey, BERAC's chair and a professor of plant sciences at the University of Missouri, Columbia. "But we can't do it at the expense of future investment."

As senior scientists receiving significant support from BER, the council members are aware of the direct impact of the cuts on their labs and their scientific productivity. Yet they seemed more worried about how others would be affected.

They fretted about graduate students and postdocs who might turn away from science after seeing the apparently random nature of federal funding. "A lot of them see what we have to do and say, 'I don't want to do that,'" said biochemist Judy Wall of the University of Missouri, Columbia. Council members also lamented the opportunities for research and education that may be lost, collaborative projects that may be hamstrung, and public outreach efforts that would be canceled or curtailed. At the same time, they recognize that scientists aren't the only ones facing hard times. Heads nodded when Stacey said: "If you think the federal government is in trouble, just look at the states. When's the last time any of us got raises?"

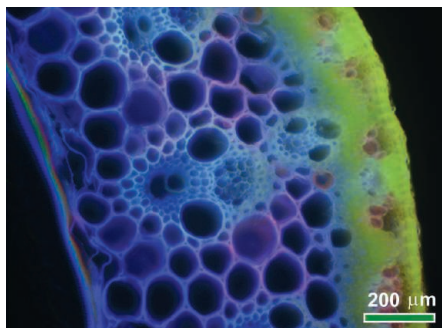
Despite the hit to their labs and their wallets, the researchers conveyed a strong sense that they had survived similar tough times in the past and expect to do likewise this time around. They even displayed a bit of gallows humor. "We've been fighting these battles for a long time," Stacey said. "So all we'll be doing is going from a bad situation to one that is intolerable." **-J.D.M.**

BER also funds an earth systems modeling program that develops and evaluates the increasingly sophisticated climate models used in the periodic reports issued by the Intergovernmental Panel on Climate Change. Many climate and environmental scientists say that BER's tandem support of modelers and model users explains why the office, among the many DOE science programs, was singled out for elimination.

"If you don't believe in climate change, why fund any research on it, including the models?" says BERAC member Judy Wall, a biochemistry professor at the University of Missouri, Columbia, who receives BER funding for research on bacteria that may be useful in bioremediation. "If you accept that climate is changing, what BER is trying to do is understand what is causing the clouds and where they occur and their optical properties, and then help to build a climate model that resolves some of these questions. And that is very useful whether you believe the temperature increases we're seeing are man-made or not."

Those who maintain that global warming is a recent scam being promulgated by radical environmentalists might be surprised to learn that BER's climate program can be traced back to DOE's Cold War forerunner, the Atomic Energy Commission (AEC). In the 1950s, AEC began funding some of the first studies of atmospheric circulation in an

attempt to understand the behavior of radioactive fallout from the nation's many nuclear explosions. That led to pioneering research in the 1970s on levels of carbon dioxide in the atmosphere. Likewise, the office's research on environmental bioremediation is also rooted in the nation's nuclear past, namely, the legacy of waste from weapons production



Inside the cell. Researchers at DOE's bioenergy research center at Oak Ridge National Laboratory hope to produce cheaper biofuels by reducing the amount of energy needed to break down plant cell walls.

and a search for environmentally friendly ways to clean up production sites.

BER has always prided itself on its ability to identify and fund interdisciplinary basic science into challenges that other agencies may be reluctant to tackle, says Aristides Patrinos, a former director of the office who is now president of Synthetic Genomics,

a San Diego, California, company started by genomics pioneer J. Craig Venter. In 1986, BER began funding the first efforts to decode the human genome, drawing upon the department's expertise with large, instrument-driven science. "We were in the bosom of the physical sciences, and their can-do, macho attitude was embedded in the culture," Patrinos recalls. He says it doesn't bother him that the National Institutes of Health and Celera Genomics, a company that Venter founded, receive the credit for completing the genome project in 2001. "That's always been our lot in life," Patrinos says. "We don't care if we get the credit. What's important is that the work got done."

BER-funded researchers don't always toil in obscurity, however. The Joint Bio-Energy Institute (JBEI) at LBNL has been something of a rock star in the biofuels community. Directed by Jay Keasling, JBEI has made significant progress in developing more efficient conversion of cellulosic biomass into liquid fuels.

That progress "would grind to a halt" under the House bill, says Keasling, a chemical engineering professor at the University of California, Berkeley, who has also achieved fame for large-scale production of synthetic artemisinin to treat malaria. Not only would the bill jeopardize BER's sizable investment, it would also trigger an immediate exodus of scientific talent. Keasling says it "would be extremely hard, if not impossible," to reassemble that expertise if BER were to be funded again in 2012.

Timothy Donohue, director of BER's Great Lakes Bioenergy Research Center in Madison, Wisconsin, says he was at an international biofuels conference in Singapore shortly after the House bill was passed and was greeted with incredulity by officials from Singapore's juggernaut Agency for Science, Technology and Research, which hosted the meeting. "Our Asian colleagues couldn't believe that the U.S. government was pulling back on its efforts to diversify the country's energy grid," Donohue says. "It didn't make any sense to them."

Donohue told them that the House bill was part of a larger debate about how best to reduce the deficit and balance the budget. But he says eliminating the bioenergy centers, and BER as a whole, doesn't seem like the right response. "My hope is that, even in difficult economic times, the country will have the foresight to continue to invest in strategies started by the previous Administration that offer the promise of strengthening our economy and creating jobs."

-JEFFREY MERVIS

Quantum Mechanics Braces for the Ultimate Test

Most accept that the quantum world is a bizarre place, but this has yet to be proved beyond all doubt. Quantum cryptography is now providing the incentive for reality's toughest test

THE 2010 SOCCER WORLD CUP IN SOUTH

Africa marked a milestone for Nicolas Gisin, although he is not a sportsman and his national team, Switzerland, did not win. A physicist by trade, Gisin views the championship with pride because it was the first international public event to employ an ultra-tight security system, devised by his Geneva-based company ID Quantique, that harnesses the weird workings of quantum physics to protect sensitive information. Now Gisin, of the University of Geneva, is on a quest to build the ultimate quantum cryptography system: one that users could trust implicitly, even if they had bought it from their worst enemy. First, however, Gisin and others have to plug a few stubborn holes in one of the bedrocks of modern physics.

Quantum mechanics is one of physics' most resounding successes, accurately describing everything from the internal workings of the atom to the structure of DNA and the makeup of neutron stars. It's spawned a wealth of technology, too, including electronics, computers, lasers, fiber optics, and nuclear power. But there's a fly in the ointment: The microscopic world that quantum mechanics describes is a bizarre place where nothing is certain and the act of observation changes things. Some physicists over the past century, including Einstein, have refused to accept that this is the only possible description of reality. Over the past 40 years, that descrip-

tion has been put to the test in a series of elegant experiments that have shown it to be true. Although most physicists find the results convincing, these experiments did skirt around a few tiny loopholes by which reality could have fooled physicists into thinking that quantum mechanics paints a complete picture.

It's these loopholes that Gisin's team and a number of other groups around the world are competing to close. The winners will have the satisfaction of settling one of the most stubborn problems in physics. As a bonus, they will also hold the key to the perfect quantum security system. "This race is on because the group that performs the first loophole-free test will have an experiment that stands in history," Gisin says.

Curiouser and curiouser

Despite its near-ubiquity in physics, quantum mechanics retains its ability to make heads spin, says Antonio Acín, a collaborator of Gisin's at the Institute of Photonic Sciences in Barcelona, Spain. Two of its most mind-scrambling features lie at the heart of quantum cryptography. The first, known as superposition, tells you that before you look, an object such as an electron can exist in two different places at the same time, or simultaneously hold two mutually exclusive properties—such as having a high or a low energy state. Only when someone measures it are the electron's multiple personalities forced to

snap into one identity, with a single location and a definite energy state. Before measurement, there's no way to predict with certainty which identity it will choose; the outcome is always random.

The second property, known as non-locality, is even stranger. It says that if, for example, two particles can be entangled—twinned together in the lab in such a way that when measured their properties correlate—then they will remain entangled even if vast distances separate them at the time of measurement. Because superposition dictates that properties don't take a fixed value until measured, one particle of the pair must somehow "know" the result of its twin's measurement. "It's as shocking as taking two dice to opposite ends of the universe and rolling them simultaneously, only to find that each time they always land on the same number," Acín says.

Toward the end of the 20th century, physicists realized that these mind-boggling properties could be harnessed to shore up the transmission of sensitive messages across the Internet. Standard cryptographic techniques work by scrambling transmissions with a secret "key"—a string of zeros and ones—that the sender and the receiver share. The key is generated by a computer algorithm, but if that is cracked, an eavesdropper can read the message.

Throwing in entanglement makes the

Long shot. Researchers beam a succession of entangled photons from a telescope in La Palma 144 kilometers to the neighboring island of Tenerife to test quantum mechanics.

eavesdropper's task much tougher, however. Suppose you entangle several pairs of particles and give both the sender and the receiver one member of each pair. Just before transmitting a message, the sender can measure the energy levels of his or her particles and assign either a zero or a one depending on the value. The resulting string of ones and zeroes can serve as a cryptographic key. By performing similar measurements on the particles' counterparts, the receiver will get an identical key, even from halfway across the universe. Because the outcome of quantum measurements can't be predicted, the key will be truly random. What's more, because quantum superpositions are disrupted whenever you look at them, any eavesdroppers trying to read the key beforehand will leave telltale evidence of their presence.

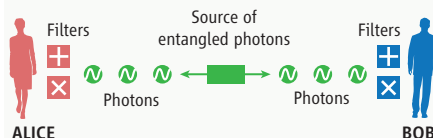
Gisin's ID Quantique is one of a handful of companies that already employ such quantum tricks in commercial applications. But Gisin and Acín want to beef up security further, producing a system so trustworthy users could buy it as a black box from a hacker and still be confident that the key it generated was secure thanks to its quantum origins. "Without that assurance, you cannot be certain that your black box isn't just spewing out a copy of a string of zeros and ones, preprogrammed by the hacker," Acín says.

Their work is based on the idea of device-independent quantum cryptography put forward in 1991 by physicist Artur Ekert, now at the Centre for Quantum Technologies in Singapore. Ekert realized that, in principle, the same tests that physicists used to prove nonlocality in the lab could be incorporated into a cryptographic system. In 2009, Gisin, Acín, and colleagues proposed a practical setup for "a box that certifies its quantum credentials at the push of a button, each time it produces a key," Acín says. Last year, Acín and colleagues took a tantalizing step toward making such a box by demonstrating that the tests could be integrated into a machine that generates random numbers using entanglement.

But the new security protocol is only as tight as the tests historically used to prove nonlocality—and that's where things get a little hairy. "Those were fantastic, beautiful experiments, but they had some shortcomings," explains Anton Zeilinger, an expert on entanglement at the University of Vienna. The tests were originally inspired by a theoretical challenge that Einstein

Ekert's Quantum Cryptography

Step 1: One photon from each entangled pair is sent to Alice and the other is sent to Bob.



Step 2: Alice and Bob choose one of two filters at random to measure the direction of polarization of each photon that they receive.

Filter choice	Possible results
Bit value assigned	0 1

Step 3: From the polarization results, Alice and Bob each generate a sequence of bit values.

ALICE						
Filter choice						
Polarization result						
Bit sequence	0	1	1	1	0	0
BOB						
Filter choice						
Polarization result						
Bit sequence	0	0	1	1	1	0

Step 4: Alice and Bob openly confer about which filters they used for each photon (but not the results). Their shared secret key is made up of the bit values generated when their filter choices matched.

Filters matched						
Shared key	0		1	1		0

Step 5: If an eavesdropper attempts to intercept any photons, the quantum correlations between the pairs are destroyed. Alice and Bob can check for this by performing a Bell test using a third filter on the photon pairs. If their correlations do not violate Bell's bound, then the system has been hacked.

threw down against quantum mechanics—but it's a challenge that, technically, has not yet quite been met.

Einstein's bugbear

Nonlocality famously galled Einstein, who derided the idea that two particles could inexplicably and instantaneously coordinate their properties as "spooky action at a distance."

In 1935, along with Boris Podolsky and Nathan Rosen, Einstein described a thought experiment that sought to show that nonlocality was absurd and quantum mechanics could never provide the final word on how the world works. Instead, he argued, the behavior of entangled particles could be explained far less mysteriously if they were preprogrammed by a set of unseen blueprints—or "hidden variables."

Einstein's position is known as local realism: Particles can't communicate instantaneously over vast distances, and their properties are real and there all the time, irrespective of measurement. Thirty years after Einstein, Podolsky, and Rosen posed their thought experiment, another physicist tried to turn it into a real one. In 1964, John Bell, a British physicist working at the CERN particle physics lab near Geneva, defined the maximum level of correlations between two entangled particles that hidden variables could explain. If a correlation exceeded Bell's limits, then local realism was violated and reality was far spookier than nonquantum physics allowed. "When I read John Bell's paper, it was like love at first sight," says Alain Aspect of the Institute of Optics in Palaiseau, France.

In the 1980s, Aspect and his colleagues set up an experiment in which pairs of photons—single particles of light—were entangled in such a way that no matter which direction they chose to measure their polarization (which could be either "parallel" or "perpendicular" to the direction of measurement), they always tallied.

Just as a police officer interrogating two suspects must keep them separated so that they do not confer, Aspect had to close any "communication loopholes" in the test. This meant ensuring that the two photons were far enough apart and that his measurements were performed fast enough that the pair could not influence each other without exchanging information faster than the speed of light, the universe's speed limit. Aspect did this by using a fast generator that changed the direction in which to measure the photons' polarizations while the photons were flying away from each other, so that they were too distant to communicate their results when the choice was made. Even with this restriction in place, Aspect found that the polarizations of the particles matched up to a degree that violated Bell's inequalities and so contradicted local realism.

The now-celebrated Aspect experiment, along with similar ones, helped to write nonlocality into physics textbooks. But there is another loophole that those experiments did

not close. The trouble is that photons are slippery customers: small, fast, and notoriously hard to detect. Typically, if five photons are hurled at a detector, it will register only one. That means that physicists can trust that Bell's bound has been violated only if they assume that the photons caught provide a fair representation of how all the photons in the experiment behaved—much the way exit polls at voting booths predict election results.

Most physicists accept that the fair-sampling assumption is a good one. "It's unlikely that nature is so malicious that it conspires with the apparatus to hold back particular photons just to fool us into thinking that quantum mechanics works," Gisin says.

Nonetheless, physicists hate loose ends, so the chase to find a perfect, loophole-free test has continued over the past decade. "Until the test is done, we can't honestly say that hidden variables have been ruled out—even if the consensus is they don't make sense—because we haven't proved it," says Harald Weinfurter of the Ludwig Maximilian University in Munich, Germany.

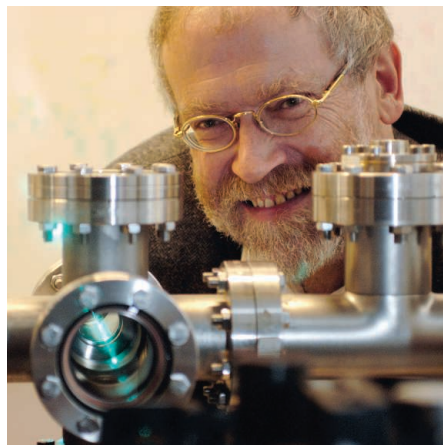
The detection loophole is also bad news for cryptographers. While it remains open, a Bell test cannot certify that a black box is working according to quantum rules. "A hacker—by definition—is malicious enough to exploit the detection loophole to fool us into thinking that a quantum process has taken place," Gisin says. As a result, Acín adds, "suddenly, this most philosophical of experiments, the loophole-free Bell test, has a practical purpose, with commercial rewards." The first group to perform it will immediately be in place to make a device-independent quantum cryptographic system.

Closing the loops

With their eyes on the prize, a group led by Paul Kwiat of the University of Illinois, Urbana-Champaign, has been collaborating with engineers at the U.S. National Institute of Standards and Technology (NIST) in Boulder, Colorado, to develop photon detectors with near 100% efficiency. "Those are good enough to perform a loophole-free test," says team member Joseph Altepeter of Northwestern University in Evanston, Illinois. The struggle now is to chain these components together with optical fibers across a large enough distance to keep the communication loophole shut. "Essentially the pieces are all in place, but the devil is in the detail," Altepeter says.

Meanwhile, Weinfurter and his colleagues are tackling the problem from an entirely different angle. They were inspired by an experiment, carried out in 2001 by

David Wineland's team at NIST, that successfully closed the detection loophole using atoms rather than photons. Because atoms are far more hefty than flighty photons, Wineland realized, they are less likely to escape the apparatus, so they provide a potentially perfect detection rate. The team performed a Bell test that compared how often the energy levels—high or low—of electrons in entangled pairs of atoms matched up. Once again, quantum mechanics was hailed victorious, as the level of correlations exceeded Bell's inequalities. But



**Earlier quantum tests
"had some shortcomings."**

—ANTON ZEILINGER,
UNIVERSITY OF VIENNA

it was not a resounding win because the atoms were close enough together to have influenced each other. In other words, the researchers had closed the detection loophole but in the process were forced to leave the communication loophole open.

Building on Wineland's experiment, Weinfurter's group is attempting to tie up both loopholes at once, by weaving photons together with atoms to reap the benefits of both. The idea is to start with two initially unentangled atoms in separate laboratories—ideally more than 100 meters apart, so that the atoms cannot influence each other over the course of the test. Each atom emits a photon; the two photons are captured and transmitted along optical fibers to a third location, where they are entangled. "The magic is that as soon as the photons are entangled, their parent atoms automatically become entangled, too," explains Weinfurter's collaborator Marek Zukowski at the University of Gdansk in Poland.

These newly entangled atoms can then take the Bell test, with a perfect detection rate, while sitting far enough apart to keep

the communication loophole closed. "The setup is being tried in two neighboring labs right now," Zukowski says. "When we are happy that everything is working, we will try it in two distant labs."

If Weinfurter can simultaneously close the detection and communication loopholes, then the verification of Bell's tests of quantum mechanics will be complete. Or will it? In the most mind-bending possible loophole of all, Bell and others have raised the possibility that experimenters may not have the free will to carry out the experiments anyway. Hidden variables, Zeilinger explains, might also be either shackling the hands of experimenters or controlling their apparatus to somehow manipulate the choice of which photon properties are measured. This could distort the results, making it appear that quantum mechanics is valid when it is not.

In a virtuoso display of long-distance entanglement, Zeilinger and colleagues ruled out this possibility. They generated entangled photon pairs at an observatory in La Palma in the Canary Islands and then fired one of them through the night sky to the neighboring island of Tenerife, where it was caught in a telescope belonging to the European Space Agency. They used random number generators to decide which measurements to make on the photons while they were in flight. But crucially, they placed a random number generator at a third, distant location on La Palma to ensure that its output could not have been influenced by hidden variables produced alongside the photons.

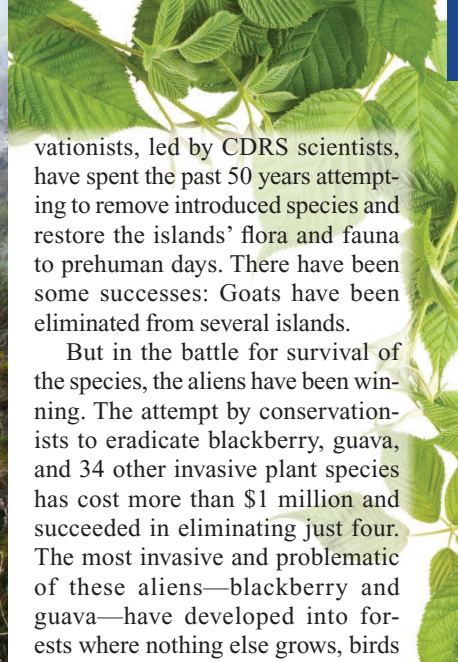
"We confirmed that Bell's limit was violated, while closing both the communication and, for the first time, the freedom-of-choice loopholes," Zeilinger says. Gisin commends the group for closing this little-known loophole. But he adds that it remains possible that hidden variables produced before the experiment began—perhaps even reaching as far back as the big bang—are predetermining all our actions. "It will be impossible to test against that type of superdeterminism," he says.

With quantum cryptography injecting momentum, Zukowski thinks the race to close all the loopholes simultaneously will soon be over. "Conservatively, it could take another 5 years to complete, but it could also be done tomorrow," he says. "We're at the stage where everyone is scared to read their competitors' papers, in case they find they have been beaten. The only real question is: Who will win?"

—ZEEYA MERALI

Zeeya Merali is a freelance writer based in London.

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CONSERVATION ECOLOGY

Embracing Invasives

The Galápagos, one of biodiversity's hot spots, has become a test case for a controversial approach to ecosystem management

GALÁPAGOS, ECUADOR—Driving uphill on Santa Cruz, one of the four inhabited islands of the Galápagos, Mark Gardener pauses to let a lumbering giant tortoise heave its 400-kilogram bulk across the tarmac. Here in the cloud forest, Galápagos finches chatter in a pale-barked, evergreen scalesia tree, its branches laden with epiphytes and lichens. Below are coastal mangroves and the golden prickly pear of the arid lowlands; in the highlands above us, the fog-laden air is wetter and the undergrowth, denser. Among a waist-high tangle of brambles and ferns, a rare rusty-leaved miconia shrub fights through, holding up its lilac flowers like a flag of defiance against the introduced weeds that threaten its once-ubiquitous existence.

Much of the fauna and flora of these islands is unique, but introduced species are taking over. These humid highlands are now a hodgepodge woodland of nonnatives, such as guava and passion fruit, and endemics, such as scalesia and guayabillo, all broken up and intruded on by agricultural plots.

Gardener has spent the past 2 decades trying to “purify” the islands’ ecosystems. But now he’s changing tack. “As scientists and conservationists, we need to recognize that we’ve failed: Galápagos will never be pristine,” says Gardener, head of restoration at the Charles Darwin Research Station (CDRS), which coordinates most of the conservation and research on the Galápagos. “It’s time to embrace the aliens.”

The sun-aged Australian researcher, with his russet beard, open sandals, and shell-pendant necklace, holds up a thorny creeper with its small, sour fruit—a transplant from Asia—and smiles sadly: “Blackberries now cover more than 30,000 hectares here, and our studies show that island biodiversity is reduced by at least 50% when it’s present. But as far as I am concerned, it’s now a Galápagos native, and it’s time we accepted it as such.”

Charles Darwin noted 17 introduced species on his visit in 1835, just 3 years after humans first started permanently living on the islands. Today, humans have intentionally or unintentionally introduced about 900 plant species into the Galápagos.

Rallying to the endemics’ cause, conser-

vationists, led by CDRS scientists, have spent the past 50 years attempting to remove introduced species and restore the islands’ flora and fauna to prehuman days. There have been some successes: Goats have been eliminated from several islands.

But in the battle for survival of the species, the aliens have been winning. The attempt by conservationists to eradicate blackberry, guava, and 34 other invasive plant species has cost more than \$1 million and succeeded in eliminating just four. The most invasive and problematic of these aliens—blackberry and guava—have developed into forests where nothing else grows, birds cannot nest, and even insects are rare. As a result, the cloud forest’s *Scalesia pedunculata*, for example, has been reduced by 97% on Santa Cruz alone.

The main reason for this failure, Gardener says, is that invasive plants are far more competitive than native plants. Seeds of invasive species, such as blackberries (*Rubus niveus*, also known as the Mysore, or hill, raspberry) are long-lived and accumulate in high numbers in the soil. “Many restoration activities fail because the disturbance they create actually stimulates these seeds to germinate, so we are stuck in a vicious cycle,” he says.

Now, Gardener is saying, enough. He points out that despite nonnative invasions, for example, the Galápagos remains one of the most pristine ecosystems left on our planet, boasting 95% of its original biodiversity. So he is joining forces with a group of maverick ecologists who for the past 5 years have promoted the idea that the addition of nonnative species to natives in a region leads to “novel” or “hybrid” ecosystems that have ecological value and may be worthy of conservation. “We need to find ways to optimize these new



Shifting ecosystems. Given the loss of the *Scalesia pedunculata* cloud forest (above and top), Mark Gardener (left) favors accepting nonnative species.

CREDITS: (TOP LEFT) NICK PATTINSON; (TOP RIGHT) ADAPTED FROM ALEKSANDR VOLKOV/ISTOCKPHOTO.COM; (BOTTOM PHOTOS) NICK PATTINSON

ecosystems,” Gardener says.

In practice, this means accepting benign species, such as banana, as “new natives.” Instead of trying to get rid of blackberry, guava, and other invasives, the new goal is simply to limit their numbers and spread so they no longer overwhelm native vegetation, possibly through biological control.

Several other places are trying to do likewise. In Panama and Puerto Rico, conservationists have decided not to fight teak and the African tulip tree, for example, but to value them as part of the changing world we live in. Next month, Hawaii joins their ranks, with a \$1.6 million grant to look at preserving native species within a hybrid ecosystem on a military reservation. In addition, the U.S. Forest Service has concluded that restoring Hawaii’s tropical forests to their historic state is “no longer financially or physically feasible.”

But Gardener’s decision to abandon the fight to preserve and restore indigenous-only species here has caused shock waves among the venerable members of the Charles Darwin Foundation, the 50-year-old organization that runs CDRS, with many of the old guard “very upset by the idea,” Gardener says. William Laurance, a conservation ecologist at James Cook University in Cairns, Australia, is also concerned: “If people want to resign themselves to managing novel ecosystems—and it sounds like that’s the reality they face on the Galápagos—then what we’re doing is homogenizing the world’s biota; setting the world on a geological epoch: the Homogocene.”

Conservation controversy

Gardener is taking his cue from ecologist Richard Hobbs of the University of Western Australia in Crawley. In 2006, Hobbs and 17 colleagues argued in *Global Ecology and Biogeography* that novel ecosystems have value in promoting biodiversity and help with services such as providing flowers for pollinators or cycling nutrients. Thus, they should be studied scientifically.

That same year, S. Joseph Wright and Helene Muller-Landau of the Smithsonian Tropical Research Institute (STRI) in Panama compared the number of species in undisturbed old-growth forest and mixed, nonnative forests in Panama. They concluded that biodiversity levels in hybrid ecosystems may be the same or actually far exceed those in comparable native forests. The finding challenged the dogma that the conversion of pris-

tine forests to novel ecosystems would necessarily lead to a swath of extinctions.

The papers provided a new perspective on what others tended to call degraded or junk ecosystems. Nonetheless, those for whom conservation means returning a forest to its “natural,” prehuman Eden were horrified. “Dr. Wright’s views have kicked off one of the most heated scientific controversies of the past decade,” Laurance says.

“I have been scolded, yelled at, and abused by the ‘conservation priests,’” says Ariel Lugo, director of the International Institute of Tropical Forestry in San Juan, Puerto Rico, and a co-author of the Hobbs paper. “Whenever I talk at a conference and give our latest results, I’m met by absolute silence and then, often, hostility from the old guard.”

Laurance, head of the critics’ camp, says the biodiversity in primary and novel eco-

And in the Galápagos, Tobias Dittmann, a botanist at the University of Hohenheim in Stuttgart, Germany, finds that this introduced tree has no negative effect on biodiversity. Dittmann looked at the biodiversity along a continuum of degraded habitats—from remnant scalesia forest to abandoned pasture—in Santa Cruz. He determined that a remnant scalesia forest has a biodiversity similar to a mixed introduced forest dominated by cedar.

There are also economic considerations in favor of these hybrid ecosystems. Gardener argues that with 30,000 people now living in the Galápagos, ecosystem planning must address human needs, such as providing timber, grazing, or shade or limiting erosion, in addition to nurturing biodiversity. Cedar is a valuable timber, bringing more than \$2 million annually to the local economy, for example. “Furthermore, coffee grown with an overstory of scalesia had an intermediate level of biodiversity,” Gardener says. “So, it seems that novel ecosystems, such as mixed introduced forest and coffee-scalesia, could be an alternative restoration objective.”

It’s still early days for this new ecology, and much is unknown. Ecosystems are naturally in a state of flux, and novel forests are just that, novel. “The oldest novel ecosystem we’ve studied in Puerto Rico is just 80 years old. That’s the time since the agricultural land was abandoned and the plants took over,” Lugo says. “So we don’t know what they will look like in 200 years’ time.”

A study in Puerto Rico suggests that although nonnatives initially dominate novel ecosystems, over time, native species establish themselves and cover a greater percentage. “Any tree that lives here has to be able to survive the once-every-60-years hurricanes that hit the islands. As a result, we see the well-adapted native trees prevailing over time,” Lugo says.

For many, abandoning the pristine dream is an acceptance of defeat, but Hobbs cautions that there is no time for sentimentality. “The conservation fraternity is still in a grieving mode because they’re seeing what’s lost,” Hobbs says. “I’m focusing on what’s there now. We have a huge opportunity to do better conservation with novel ecosystems, because whatever the future looks like, it will be very different from the past.”

Here in the Galápagos, Gardener hopes to have a hand in deciding what that future looks like.

—GAIA VINCE

Gaia Vince writes on environmental issues in the developing world at wanderinggaia.com.



Alien invader. Blackberry’s carpet of brambles has overwhelmed native ecosystems on the Galápagos.

systems is not equivalent because the latter have more “junk species,” which thrive in many places. “We need to protect the rare plants and animals that are found in primary forests,” he argues. In many comparative biodiversity studies, novel ecosystems do well only because of their proximity to pristine forests, he cautions: “The weedy exotics get a subsidy of immigrants from primary forest, which boosts their diversity figures.”

The controversy came to a head in 2008 during what Lugo describes as a “highly volatile” meeting held at STRI. The outcome was a grudging acceptance that secondary forests do have conservation value. Nonnatives can help a heavily impacted area regain its diversity by creating canopy, stabilizing the soil, or retaining moisture. Thus endemic plants, which often take longer to reestablish themselves on former pasture or at the edges of agricultural land, can benefit from the shade offered by faster-growing, nonnative trees, like cedar.



CONSERVATION ECOLOGY

Lost Causes Get New Hope

A commitment to “zero extinction” shines a spotlight on the world’s most endangered species

DOGO ISLAND, JAPAN—For most of his life, Masaru Notsu has shared a small round island in the Sea of Japan with the Oki salamander (*Hynobius okiensis*), a critically endangered species found nowhere else on Earth. And for most of his life, the naturalist has watched plantations, erosion-control dams, and forestry roads chip away at the amphibian’s 10-square-kilometer habitat. “This valley was their paradise,” says Notsu, standing at the bottom of a yawning gneiss quarry where a spawning stream once flowed. “In the space of 30 years, it’s been destroyed.”

Nobody knows how many Oki salamanders are left. Saving the palm-sized forest dweller from extinction could require habitat restoration, stricter conservation laws, and a revolution in how island residents approach development. Proponents of conservation triage might question the cost—but a new global commitment to zero extinction should give a major boost to the Oki salamander and other species in need of a lifeline.

Last October, the 193 parties to the Convention on Biological Diversity drew up a new plan to stem biodiversity loss. In the plan’s Target 12, countries pledged to prevent the extinction of known threatened species and improve their conservation status, especially those in steepest decline, by 2020. “It’s the most ambitious thing to come out of the meeting,” says Conservation International President Russell Mittermeier, whose organization, based in Arlington, Virginia, pushed hard for the pledge. “Target 12 gives everyone in conservation an official mandate,” adds Jean-Christophe Vie of the International Union for the Conservation of Nature (IUCN) in Gland, Switzerland. “You don’t have to argue with countries; you can point to the document they all agreed to.”

Critics say Target 12 is misguided. “It’s

like spending all your money in the health system on 85-year-old people who smoked their whole life and need lots of multiple bypasses and none on preventative medicine,” says Hugh Possingham, a mathematical ecologist at the University of Queensland in Brisbane, Australia, who models how limited budgets can be used to save the most species. With resources for conservation in short supply, Possingham argues, planners must consider each project’s cost and the likelihood of success or risk neglecting projects that could have a bigger long-term impact.

Despite such qualms, the Target 12 mandate promises to shape funding decisions for years to come. The World Bank and the Global Environment Facility—which together provide about 60% of biodiversity-related aid—have signed on. They have pledged to support developing countries that wish to protect the sole remaining habitats of endangered plants and animals at sites compiled by the Alliance for Zero Extinction (AZE), a coalition working to identify IUCN Red List endangered and critically endangered species that are confined to one location. The Oki salamander is one of 920 species at 587 AZE sites. Brazil and several countries have begun using AZE’s map to set conservation priorities.

Backers insist that Target 12 will not bleed worthier causes. Preventing imminent extinction is “the most important thing you can spend biodiversity money on,” asserts AZE chair Michael Parr, vice president of the American Bird Conservancy in Washington, D.C. “It’s also relatively inexpensive because the total land area for these [AZE] sites is comparatively small.”

Target 12 does have its limits. Untold numbers of embattled species haven’t made it onto the Red List—and are left out of zero-extinction efforts. “Focusing on imminent



Zeroing in. Naturalist Masaru Notsu (top) has spent a lifetime observing the Oki salamander, found only on Dogo Island off Japan’s west coast. The amphibian is a target of a “zero extinction” drive.

extinctions is necessary but not at all sufficient for biodiversity,” says Taylor Ricketts, head of WWF’s Conservation Science Program and lead author of a 2005 paper introducing the AZE mapping project. “I worry that too much focus on AZE could distract us from the vast majority of species that haven’t been assessed.”

Still, AZE’s imprimatur can give a species a shot in the arm. After the Oki salamander’s AZE listing, the Shimane Prefecture government launched a formal survey last year of the poorly studied creature, and the town banned its collection and sale. The next question is whether the Japanese government will push adherence to Target 12 at the local level. Although authorities on job-strapped Dogo Island have begun to emphasize ecotourism over resource extraction, Notsu is skeptical they would turn down construction projects to protect salamander habitat. “Can we eat it or make money off it? That’s the usual question,” he says.

Such concerns are expected to crop up all over AZE’s map. Conservationists must have a persuasive response. “It’s not simply a matter of putting some money on the table and saying ‘don’t destroy these areas,’ because that just doesn’t work. It’s really about protecting them as a part of a broader development and poverty-reduction plan,” says James Warren Evans, director of the World Bank’s Environment Department. Target 12 is a call to arms against the extinction threat; it will be up to stakeholders to make sure treaty parties show up on the battlefield.

—WINIFRED BIRD

Winifred Bird is a writer in Japan.

CREDITS (CLOCKWISE FROM LEFT): MASARU NOTSU; W. BIRD; M. TWOMBLY/SCIENCE

LETTERS

edited by Jennifer Sills

Creating a Buzz About
Insect Genomes

WHEN E. O. WILSON PROCLAIMED THAT INSECTS ARE THE “little creatures who run the world” (1), he was simply reaffirming the long-recognized dominance of the largest class of animals on our planet. Insects constitute approximately 53% of all living species, with one group alone (the ants), accounting for almost a quarter of terrestrial animal biomass (2). These tiny creatures also exert outsized impacts on human affairs. By serving as pollinators to more than 75% of flowering plant species (3), insects are essential to the maintenance and productivity of natural and agricultural ecosystems. But other insects consume or damage more than 25% of all agricultural, forestry, and livestock production in the United States, costing our economy more than \$30 billion annually (4). These losses occur despite more than 150 years of concerted efforts to prevent them. Insects and other arthropods not only affect our food supply, they also carry disease. Parasites and pathogens carried by insects and their relatives have led to more loss of human life than all wars in recorded history; even today, insect-borne diseases are a leading cause of death of children under the age of 5 (5). The annual cost of vector-borne diseases worldwide is estimated at almost \$50 billion (6). Clearly, our health and well-being depend on our ability to understand and manage arthropods of agricultural, medical, and veterinary importance.

In the past decade, biomedical research has increasingly relied on information obtained from sequencing the human genome, and early genome-enabled successes have inspired a new vision of genomic medicine (7). We believe that genomics also can improve our lives by contributing to a better understanding of insect biology and transforming our ability to manage arthropods that threaten our health, food supply, and economic security. Because of the overwhelming diversity and abundance of insects, achieving these goals will require a project of grand scale.

Therefore, we, the undersigned, are pleased to announce the launch of the “i5k” initiative to sequence the genomes of 5000 species of insects and other arthropods during the next 5 years (8). This project is aimed at sequencing and analyzing the genomes of all species known to be important to worldwide agriculture and food safety, medicine, and energy production; all species used as models in biology; the most abundant insects in world ecosystems; and, to achieve a deep understanding of arthropod evolution, representatives of insect relatives in every major branch of arthropod phylogeny. The i5k initiative will be broad and inclusive, seeking to involve scientists from around the world and obtain funding from academia, governments, industry, and private sources. We also aim to encourage new collaborative research by computer scientists, bioinformaticians, and biologists to overcome the challenges of handling



this unprecedented volume of data and derive meaning from these genomes.

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8. We invite individuals to suggest species for sequencing and to alert us of pending and ongoing insect genome projects. Please visit www.arthropodgenomes.org/wiki/i5K to provide your input.

Letters to the Editor

Letters (~300 words) discuss material published in *Science* in the past 3 months or matters of general interest. Letters are not acknowledged upon receipt. Whether published in full or in part, Letters are subject to editing for clarity and space. Letters submitted, published, or posted elsewhere, in print or online, will be disqualified. To submit a Letter, go to www.submit2science.org.

Genomic Medicine: The Social Science View

IN THE NEWS FOCUS STORY “WAITING FOR the revolution” (4 February, p. 526), E. Marshall summarizes roadblocks to an anticipated genomic medicine revolution. However, his emphasis on the lack of proven clinical benefits may be misleading. Physicians do adopt technological innovations, even when superior clinical utility has not been proven, when they feel a

moral imperative to intervene in the face of life-threatening illness, or when they are pressured by patient advocacy groups and/or industry (1). Genomic medicine is progressing most in areas with substantial benefits in terms of lives saved and treatments avoided: cancer diagnostics and therapeutics, improved management of some disease subtypes (such as MODY diabetes), and some applications of pharmacogenomics.

Rather than focusing on physician knowledge deficits and technological fixes such

as automated aids to data sourcing, I would argue instead for more emphasis on understanding sociological factors involved in medical innovation and clinical uptake. Such analyses should draw from the investment that a number of governments have made over the past decade in the social science of genomics (2). A review of the missing revolution in genomic medicine would look quite different written from a social science perspective.

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Genomic Medicine: Putting Our Tools to Use

IN THE NEWS FOCUS STORY “WAITING FOR THE revolution” (4 February, p. 526), E. Marshall discussed the use of genetic diagnostics in medicine. However, an analysis of the medical genetics revolution should focus not only on diagnostics but also on genetic manipulation.

The genetic revolution is well under way in microbes and invertebrates. It's even enjoying great progress in mice and moderate progress in rats. The real progress in these organisms has come not from the ability to read their genes; rather, it is a result of our ability to write them.

Improving modern treatments, including pharmaceuticals, based on genetic differences is certainly a valuable addition to our medical toolbox. But I predict that the advent of genetic manipulations in humans—whether in embryos or in adults, whether transient or permanent, whether purely genetic or epigenetic—will be considered the real beginning of the genetic revolution.

Before we proceed down this road, we must address numerous risks and ethical questions. But we cannot experience any sort of genetic revolution in human medicine until we push through these obstacles and address the benefits to humanity of repairing the aberrantly expanded HTT gene in a middle-aged Huntington's patient or knocking in a copy of the CFTR gene in a child suffering from cystic fibrosis. Diagnostics are just viewing the toolbox through a window. Eventually, we'll need to figure out how to safely pick up the tools and make good use of them.

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CORRECTIONS AND CLARIFICATIONS

Reports: “Atmospheric CO₂: Principal control knob governing Earth's temperature” by A. Lacis et al. (15 October 2010, p. 356). The legend for Fig. 1 should have cited a paper by the authors that was also published in October 2010: G. A. Schmidt et al., *J. Geophys. Res.* **115**, D20106, 10.1029/2010JD014287 (2010). The *Science* Report included better statistical weighting; the uncertainty limits were more precise for the (instantaneous) greenhouse effect fractional response attribution shown in Fig. 1. For additional comparison, greenhouse effect attribution based on one-dimensional model adjusted forcing formulas [given in J. Hansen et al., *J. Geophys. Res.* **93**, 9341 (1988)] yields fractional responses of 0.210, 0.014, and 0.011 for 1980 atmospheric amounts of CO₂, CH₄, and N₂O, respectively, in close agreement with the fractional responses shown in Fig. 1 and the tabulated values in table S1 of the Supporting Online Material. The Hansen et al. study provides additional corroboration for the robustness of the radiative transfer–based attribution of the terrestrial greenhouse effect.

TECHNICAL COMMENT ABSTRACTS

Comment on “Does the Hydrated Electron Occupy a Cavity?”

László Turi and Ádám Madarász

Larsen et al. (Reports, 2 July 2010, p. 65) challenged the long-standing model of the solvent geometry surrounding a free electron in water using molecular dynamics simulations based on a newly derived electron-water pseudopotential. We illustrate that, in contrast to the model they used, the true electron-water interaction is repulsive in the region relevant to the reported extended electron distribution, consistent with the cavity model.

Full text at www.sciencemag.org/cgi/content/full/331/6023/1387-c

Comment on “Does the Hydrated Electron Occupy a Cavity?”

Leif D. Jacobson and John M. Herbert

Larsen et al. (Reports, 2 July 2010, p. 65) suggest that, contrary to the established paradigm, the aqueous electron does not carve out and occupy a cavity in liquid water. Closer examination of their theoretical model, however, reveals that many of its predictions differ substantively from established benchmarks and that its behavior differs qualitatively from Hartree-Fock theory, upon which the model is based.

Full text at www.sciencemag.org/cgi/content/full/331/6023/1387-d

Response to Comments on “Does the Hydrated Electron Occupy a Cavity?”

Ross E. Larsen, William J. Glover, Benjamin J. Schwartz

Turi and Madarász and Jacobson and Herbert argue that the pseudopotential we derived for the hydrated electron contains inaccuracies that make it overly attractive. We show that our potential is derived correctly and argue that the criticisms presented are not relevant when evaluating a pseudopotential's accuracy for condensed-phase simulation. Neither critique addresses our central result that the experimental properties of the hydrated electron are consistent with a noncavity picture.

Full text at www.sciencemag.org/cgi/content/full/331/6023/1387-e

Comment on “Does the Hydrated Electron Occupy a Cavity?”

László Turi* and Ádám Madarász

Larsen *et al.* (Reports, 2 July 2010, p. 65) challenged the long-standing model of the solvent geometry surrounding a free electron in water using molecular dynamics simulations based on a newly derived electron-water pseudopotential. We illustrate that, in contrast to the model they used, the true electron-water interaction is repulsive in the region relevant to the reported extended electron distribution, consistent with the cavity model.

The paper by Larsen, Glover, and Schwartz (LGS) claimed that an alternative physical picture of the hydrated electron model emerges from a series of mixed quantum-classical molecular dynamics simulations based on their new model (*1*). At the heart of the claim is the development of a new electron-water molecule pseudopotential by these authors that describes the interaction between the classically treated water molecules and the quantum mechanical excess electron. The authors correctly state that “A key element in any simulation of the hydrated electron is the electron-water interaction specified by the pseudopotential.” In this comment, we inspect the pseudopotential of LGS and show that inaccuracy in their description of the core region of the potential seriously undermines the credibility of the subsequent simulation results.

The following analysis applies protocols based on quantum mechanical model calculations similar in spirit to previous studies (*2, 3*). We solve the Schrödinger equation for the excess electron in the field of the Hartree-Fock potential of a single water molecule (*4*) and a conveniently chosen repulsive potential (*5*). The repulsive potential confines the electron in the vicinity of the water molecule (here within an approximate sphere of ~ 5 Å radius) and makes it possible to test the pseudopotential in the chemically most relevant region. The results of the exact calculations are compared with those using the reported pseudopotential (*1, 4*), and also to a reference system of a free electron placed in the same repulsive potential. Because the energy and the electron density of the excess electron are sensitive to changes in the underlying potential, the comparison helps to pinpoint problems in the LGS pseudopotential.

The ground-state energy for the reference system, a free electron confined by the repulsive potential, is 4.6585×10^{-2} hartree (*5, 6*). The exact solution of the excess electron Schrödinger equation, in the field of the water wave function and the confining potential, results in 5.0257×10^{-2} hartree. The

more positive eigenenergy in the presence of the water molecule immediately indicates that the average potential felt by the electron is repulsive in the vicinity of the water molecule. Although the difference in the energies is only ~ 0.1 eV, the impact of the underlying repulsion becomes important in condensed-phase simulations, as illustrated below. Replacing the exact electron-water potential by a rigorously derived pseudopotential in the Hamiltonian, one should obtain the same eigenenergy. The LGS potential, however, gives 4.5725×10^{-2} hartree (*4*). The lower energy relative to the reference system unequivocally confirms that instead of reproducing the average repulsion of the exact electron-water potential, the LGS potential is slightly attractive in the vicinity of the water molecule. The LGS model also fails to reproduce the electron density of the exact pseudo-wave function (*7*) (Fig. 1A). The considerably increased electron density on the oxygen side of the molecule by the LGS model relative to the exact density indicates that the LGS potential artificially introduces attraction here, whereas the depleted electron density on the hydrogen side suggests an opposite deviation, decreased attraction. The net result is the artificially attractive LGS potential. As directly related symptoms, suspicious regions appear in the reported pseudopotential that are not consistent with basic physical intuition. These include a relatively deep (~ 0.5 eV) attractive well in the molecular plane on the generally repulsive oxygen side of the molecule at ~ 3 Å from the oxygen and a repulsion in the generally attractive dipole direction on the hydrogen side at ~ 4 Å (see the inset of Fig. 1B). At these distances, one would expect simple electrostatics to dominate. These features are not shown in figure 1 in Larsen *et al.* (*1*).

To demonstrate the serious consequences of an incorrect description of the core region in the LGS model, we performed quantum molecular dynamics simulations of a quantum mechanically treated electron in a classical water bath of 499 molecules, identical in setup to that described in (*1*). In the simulation, we employed a modified LGS parameter set chosen by optimizing one parameter on the repulsive oxygen center in the original LGS model to reproduce the computed

exact model eigenenergy (*8*). This modification is minor (Fig. 1B), similar in magnitude to the uncertainty of the numerical fitting of the LGS potential on the exact smoothed potential (*1, 9*). The correction is nevertheless manifest in dramatically different properties of the hydrated electron compared with those of LGS (*1*). Most notably, the radial distribution functions testify to the formation of a solvent cavity (Fig. 1C) with a well-defined excluded volume around the electron (~ 0.5 Å radius for the hydrogen and ~ 1.5 Å radius for the oxygen), in sharp contrast to the LGS model. In parallel, the radius of the electron collapses to 2.3 Å from the LGS value of 2.6 Å, indicating a more compact electron distribution. The vertical detachment energy (VDE) of the hydrated electron also undergoes a considerable change. The LGS potential predicts a VDE that is much higher [≥ 5 eV (*1*)] than experimental values of 3.3 eV (*10*) and 3.6 eV (*11*) or the ~ 4 eV simulation result using a pseudopotential consistent with a cavity-based model (*12*). The simulation with the modified potential brings the VDE closer to the consensus range by 0.85 eV.

We have demonstrated that the electron-water pseudopotential of Larsen *et al.* (*1*), although based on a rigorously derived potential surface, contains inaccuracies (*13*). The simulated properties of the hydrated electron are shown to be very sensitive to this problem. A simple introduction of a modified repulsion (by matching the exact energy of the quantum model) has been demonstrated to recover the traditional cavity picture, consistent with other pseudopotentials (*3, 14, 15*). The physical properties of the hydrated electron by Larsen *et al.*, simulated on the incorrect potential, and the new structural characterization in particular, appear to be artifactual. Finally, we note that this cavity model of the hydrated electron is in accord with extensive, model-free ab initio molecular dynamics simulations (*16*).

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4. The model does not contain the polarization potential in the present approximation (*2, 3*). The same scheme was used at the development of the LGS potential (*1*). The full potential, including the a posteriori introduced polarization potential, is used in the molecular dynamics simulations.
5. The repulsive potential, $V_{\text{conf}} = \frac{1}{2}k(x^8 + y^8 + z^8)$ where $k = 10^{-8}$ atomic units, is centered on the water molecule center of mass (*2, 3*). The repulsive potential hardly perturbs the potential energy surface at and around the water molecule, but erects a steep repulsive wall at ~ 5 Å from the water molecule center of mass.
6. In the calculations, we used the same water geometry as Larsen *et al.* (*1*). The water wave function is evaluated at HF/6-31++G(d,p)+aug level (*2, 3*). The basis set representing the excess electron consists of 86 Gaussian lobe functions [transformed from the atom-centered 6-31++G(d,p)+aug basis set of the water molecule] and $7 \times 7 \times 7$ s-type functions (exponent 0.03) evenly distributed on a cubic grid of 20 bohr length centered on the water molecule (*2, 3*). We verified that the calculations are converged with respect to the size of the distributed basis set.

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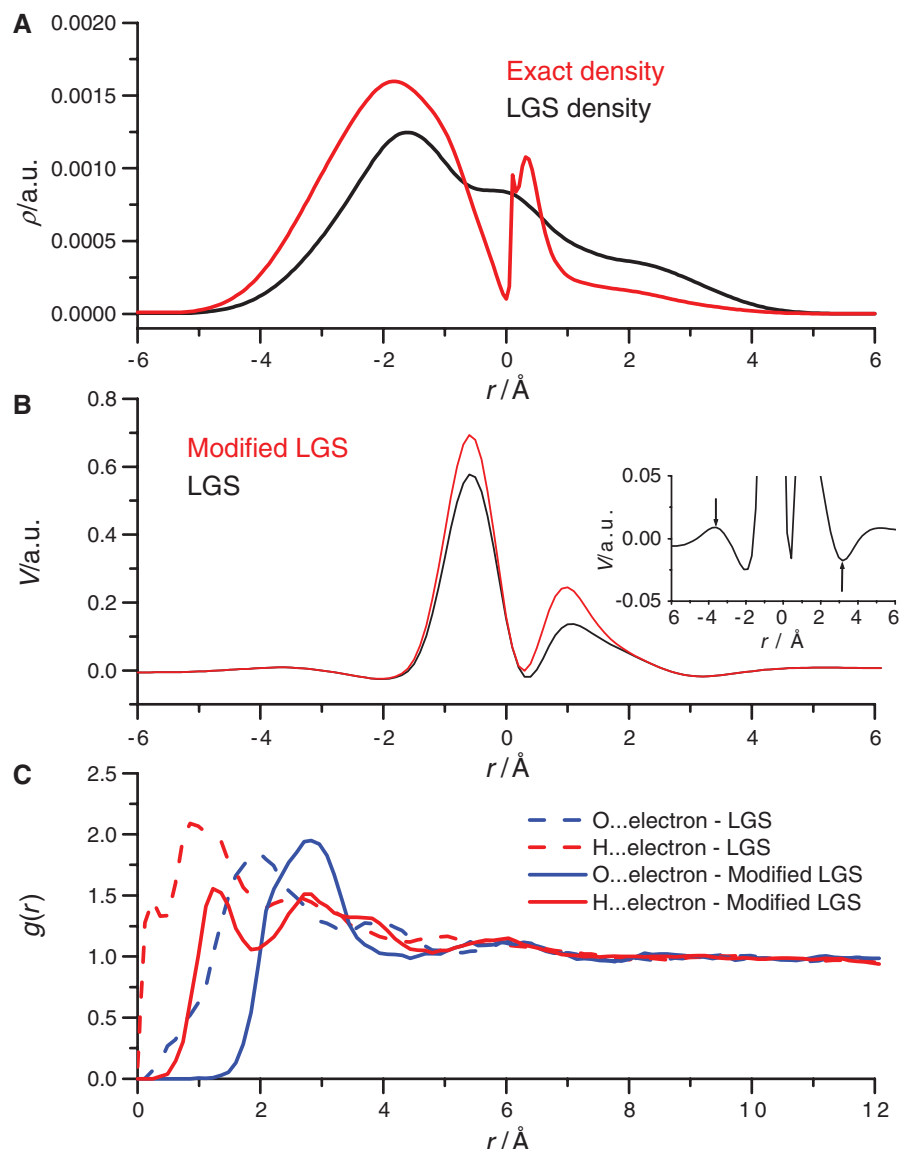


Fig. 1. (A) The electron density of the exact pseudo-wave function (red) and the LGS electron density (black) along the dipole direction in the molecular plane through the oxygen atom. The center of mass of the water molecule is at the origin; the hydrogen atoms are at negative coordinates. (B) The repulsion modified pseudopotential (red) in the same molecular direction as above. For comparison, the original LGS potential is also shown (black). The arrows in the inset show suspicious parts of the LGS potential. For clarity, the potentials do not contain the additive attractive polarization contributions. (C) Electron-hydrogen (red) and electron-oxygen (blue) radial distribution functions using the modified pseudopotential including polarization (solid curves). For comparison, the radial distribution functions obtained with the LGS potential (dashed) are also shown.

7. The pseudo-wave function was calculated as in (1) and (2).
8. We changed the parameter B_2 from its original value of 0.209915 hartree to 0.34 hartree. The eigenenergy of the quantum model calculation changes from 4.5725×10^{-2} hartree to 5.0236×10^{-2} hartree. The exact value is 5.0257×10^{-2} hartree. The modified set keeps the qualitative shape of the LGS potential.
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Comment on “Does the Hydrated Electron Occupy a Cavity?”

Leif D. Jacobson and John M. Herbert*

Larsen *et al.* (Reports, 2 July 2010, p. 65) suggest that, contrary to the established paradigm, the aqueous electron does not carve out and occupy a cavity in liquid water. Closer examination of their theoretical model, however, reveals that many of its predictions differ substantively from established benchmarks and that its behavior differs qualitatively from Hartree-Fock theory, upon which the model is based.

A recent report by Larsen, Glover, and Schwartz (1) (LGS) challenged the long-held view that the “hydrated” (aqueous) electron, e_{aq}^- , consists of a one-electron wave function localized within a quasispherical solvent cavity and coordinated to four to six water molecules. This has been the dominant paradigm for more than 40 years (2), and it is supported by numerous atomistic simulations (3–5). The challenge by LGS is based on a new, “rigorously derived” electron-water pseudopotential, and simulations using this one-electron pseudopotential model do not afford a

well-defined cavity. This model, however, has not yet been tested against reliable benchmarks. Such tests are reported here.

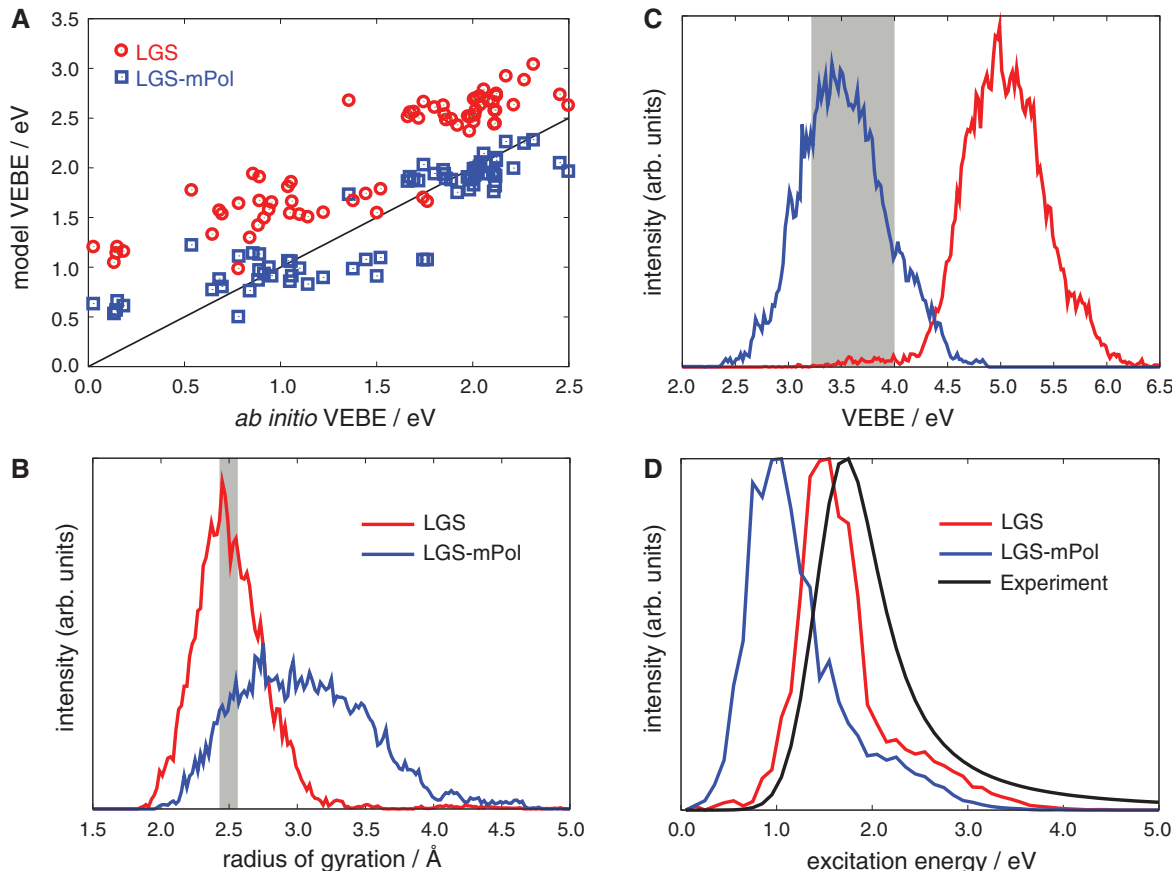
The LGS pseudopotential (1) is derived within the static exchange (SE) approximation, which essentially amounts to a Hartree-Fock calculation for H_2O^- using frozen molecular orbitals (MOs) for H_2O . LGS have devised a clever way to obtain a nodeless pseudo-orbital for the unpaired electron without introducing approximations that are typically made in this context (6). Once the pseudo-orbital is determined, it can be converted into an electron-water pseudopotential for condensed-phase simulations.

The LGS pseudopotential was fit using Mulliken atomic charges $Q_O = -0.862709e$ and $Q_H = +0.431355e$ obtained from a Hartree-Fock

calculation, but e_{aq}^- simulations were performed using $Q_O = -0.82e$ and $Q_H = +0.41e$, corresponding to the simple point charge (SPC) water model (1). This corresponds to a reduction in H_2O dipole moment from 2.39 D to 2.27 D, at the SPC geometry. LGS augmented their model with an approximate polarization potential, which is not included in the SE treatment, and far less attention is paid to this aspect of the model. Polarization parameters from the literature were used, without further comment.

High-level ab initio calculations have been reported for $(H_2O)_n^-$ clusters, for $n = 2$ to 33 (7), but LGS did not report any comparisons to these data. Figure 1A compares benchmark ab initio vertical electron binding energies (VEBEs) to results obtained using the LGS pseudopotential. The LGS model strongly overbinds the electron compared with ab initio calculations. By modifying a single parameter in the polarization potential (R_c) [see supporting online material for (1)], we obtain a model that we call LGS-mPol that performs reasonably well against this benchmark database, albeit not as well as other hydrated-electron models in the literature (4, 5, 7). We do not intend LGS-mPol to be a serious e_{aq}^- model, but rather to demonstrate that the overbinding exhibited by the LGS model cannot be fixed in a simple way, without deleterious effects on other observable properties.

Fig. 1. (A) Comparison of MP2/6-31(1+,3+)G* benchmark VEBEs (7) to results obtained using LGS-based pseudopotential models, for 71 different $(H_2O)_n^-$ isomers ranging from $n = 12$ to $n = 33$. (B to D) Radius of gyration, VEBE, and optical absorption spectrum of e_{aq}^- , obtained from bulk simulations. The gray boxes in (B) and (C) depict the range of the experimental estimates of these quantities (8–12).



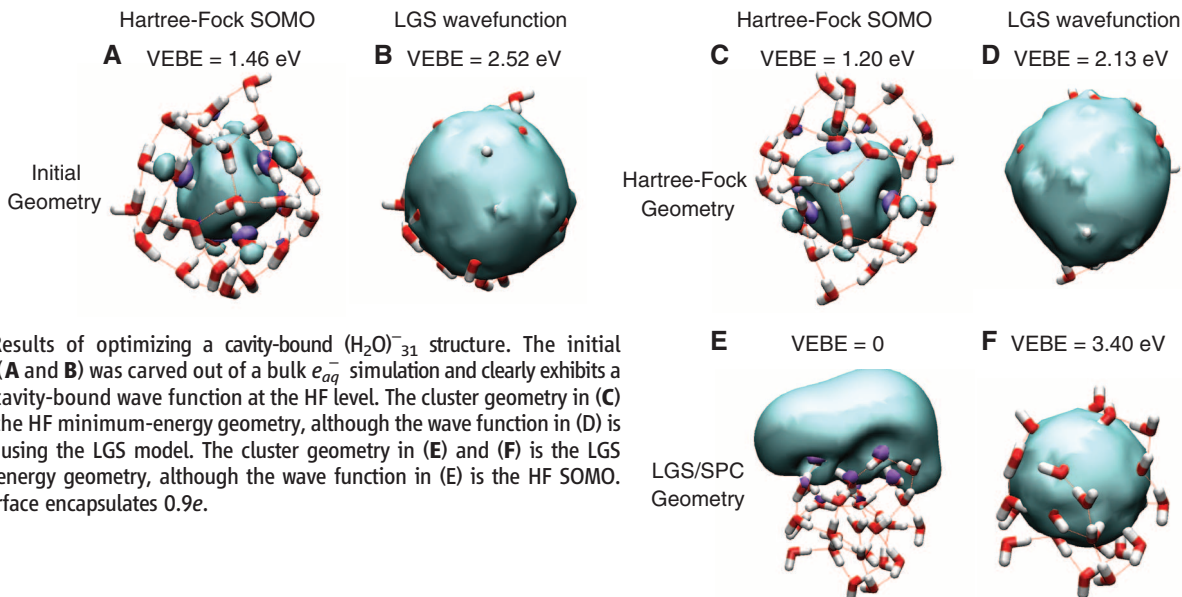


Fig. 2. Results of optimizing a cavity-bound $(\text{H}_2\text{O})_{31}^-$ structure. The initial geometry (**A** and **B**) was carved out of a bulk e_{aq}^- simulation and clearly exhibits a compact, cavity-bound wave function at the HF level. The cluster geometry in (**C**) and (**D**) is the HF minimum-energy geometry, although the wave function in (**D**) is calculated using the LGS model. The cluster geometry in (**E**) and (**F**) is the LGS minimum-energy geometry, although the wave function in (**E**) is the HF SOMO. Each isosurface encapsulates $0.9e^-$.

We have performed molecular dynamics simulations of e_{aq}^- using the same simulation procedure reported in (1). We find that radial distribution functions and other structural properties reported by LGS are not strongly affected by variation of the polarization potential or the H_2O point charges. Rather, it is the LGS pseudopotential itself that is indisposed toward cavity formation.

Figure 1, B to D, shows several properties obtained from our simulations and compares them to experimental estimates. The average radius of gyration for the LGS model is in good agreement with experiment (8), but the agreement is far less satisfactory for the LGS-mPol variant examined here, which performs much better for VEBEs. The optical absorption spectrum predicted by the LGS model is slightly red-shifted, relative to experiment, and a more rigorous treatment of solvent polarization should further red-shift the spectrum (5). The attenuated polarization potential in LGS-mPol yields a spectrum that is red-shifted from experiment by 0.7 eV, which makes sense given the larger radius of gyration predicted by this model.

Strikingly, all 29 excited states that we used to generate absorption spectra are vertically bound. In fact, the 29th excited state is bound, in the LGS model, by ~ 1.0 eV. Figure 1C plots the distribution of ground-state VEBEs obtained from the simulations. The LGS model overbinds the electron by 1 to 2 eV, whereas LGS-mPol predicts a VEBE within the range of experimental estimates for e_{aq}^- (8–12). This agreement is partly fortuitous, as the binding energy increases by ~ 1 eV if Ewald summation is used to sum the long-range Coulomb interactions (5), whereas we followed the procedure of LGS (1) and used the minimum-image convention.

Larsen *et al.* (1) plotted the ground-state e_{aq}^- energy as a function of time. This function oscillates around -5.5 eV, whereas the lowest few excited-state energies oscillate between -3 eV and

-4 eV. In contrast, the ground-state energy inferred from experiment is 3.4 to 4.0 eV (8–12). In view of this tremendous discrepancy, the assertion by LGS that “in every case...our predictions are consistent with experiment” appears to be overstated.

Ab initio calculations on $(\text{H}_2\text{O})_n^-$ clusters find that orbital relaxation upon electron detachment is fairly minor (13); hence, the Hartree-Fock (HF) singly occupied MO (SOMO) should offer a qualitatively correct description of the unpaired electron, provided that this orbital is bound. The LGS pseudopotential, in conjunction with an ad hoc polarization potential, is intended to mimic the HF SOMO. To examine the extent to which it does so, we carved out a $(\text{H}_2\text{O})_{31}^-$ cluster from a cavity-forming model of e_{aq}^- (4), which represents a 5.5 Å radius around the centroid of the cavity-bound wave function. The geometry of this cluster was subsequently optimized using HF/6-31++G* theory and, alternatively, the LGS model. Figure 2 shows that HF optimization preserves the cavity-bound nature of the SOMO, but this cavity collapses when optimized using the LGS model. The latter affords a wave function that permeates throughout the cluster. At the LGS-optimized geometry, the HF SOMO is unbound and is localized on the surface of the cluster; it has been “squeezed out” by the collapse of the cavity.

Collapse of the solvent cavity arises because the LGS pseudopotential is far more attractive near the hydrogen atoms than previous pseudopotentials. This feature results from the fact that the density associated with the “exact” pseudo-orbital obtains a maximum over the hydrogen atoms, whereas the density of the exact SE wave function is at a minimum [see figure 1 in (14)]. Any pseudopotential derived from this pseudo-orbital will result in a far-too-attractive region near the hydrogen atoms. In contrast to the LGS potential, the true HF potential is clearly repulsive in these regions, as evidenced by the “dents”

in the HF SOMO around each water molecule (see Fig. 2).

The structure of e_{aq}^- is intimately quantum-mechanical, and cannot be probed directly by experiment, so there is an acute need for theoretical models to aid in the interpretation of experimental observables. Before new theoretical predictions can be taken seriously, however, such models must be carefully tested against the large body of existing e_{aq}^- data. Relative to the current generation of cavity-forming pseudopotential models (4, 5), the model introduced by Larsen *et al.* (1) fares poorly in such tests.

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Response to Comments on "Does the Hydrated Electron Occupy a Cavity?"

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Turi and Madarász and Jacobson and Herbert argue that the pseudopotential we derived for the hydrated electron contains inaccuracies that make it overly attractive. We show that our potential is derived correctly and argue that the criticisms presented are not relevant when evaluating a pseudopotential's accuracy for condensed-phase simulation. Neither critique addresses our central result that the experimental properties of the hydrated electron are consistent with a noncavity picture.

In our original paper (*1*), we derived a pseudopotential for the hydrated electron to represent its condensed-phase behavior. In their critiques, Turi and Madarász (*2*) and Jacobson and Herbert (*3*) argue that our pseudopotential contains inaccuracies that make it overly attractive, such that the noncavity nature of the hydrated electron we reported is unphysical. Here, we note that the derivation of our potential is correct. We also argue that the criteria by which Turi and Madarász and Jacobson and Herbert criticize our potential, which are based on the vertical electron binding energy (VEBE) of an excess electron interacting with either a single water molecule or small clusters, are not relevant for evaluating the quality of a pseudopotential for condensed-phase simulation. Finally, we point out that neither comment addresses the central claim of our paper, which is that our results demonstrate that the known experimental properties of the hydrated electron are consistent with a noncavity picture.

To derive our pseudopotential, we started with a Hartree-Fock (HF) calculation on a single water molecule and determined the lowest unoccupied molecular orbital (LUMO). We then used this LUMO to calculate the Phillips-Kleinman (PK) pseudo-orbital, a wave function that is constructed to be nodeless, to match the LUMO outside the water core molecular orbitals, and to have the same eigenenergy as the LUMO (*4, 5*). Our pseudopotential was then rigorously determined as that whose one-electron Schrödinger equation has the PK pseudo-orbital as its ground state (*4*).

When all of the electrons are confined by an external potential, however, the LUMO one obtains is different from that of an unconfined system, so the resulting pseudopotential is also different. Thus, it is not strictly correct for Turi and Madarász (*2*) to compare their pseudo-orbital, which was calculated using a LUMO with a confining potential, to that generated from our potential, which was based on the unconfined LUMO. Nevertheless, as shown in figure 1A of their comment [and also figure 2A in

(*6*)], whether or not there is a confining potential, the water pseudo-orbital has features with high electron density near the oxygen atom, which create attractive regions in the PK pseudopotential that reproduces this density. Moreover, to use our pseudopotential in condensed-phase calculations, we smoothed our pseudo-orbital at the resolution of the grid basis set used in our MD simulations (*1*). Thus, much of the apparent discrepancy in figure 1A of Turi and Madarász (*2*) results from their choice to compare the raw, unsmoothed PK pseudo-orbital to that reproduced by our potential based on a smoothed pseudo-orbital.

Once we produced our numerically exact, smoothed pseudopotential, we followed the standard approach of fitting it to an analytic function. We could have constrained our fit to guarantee that the resulting analytic potential generated the original eigenenergy, even if this caused the fit to miss some of the physical features of the potential, as has been done by others (*6, 7*). Instead, we chose a function that best represented the physical features of the potential (*1*), even though this had the numerical consequence of slightly altering the single-molecule eigenenergy. We took this approach because we felt that it was important to account for the large attractive and (off-atom) repulsive features that have not been included in previous water-electron pseudopotentials. Thus, Turi and

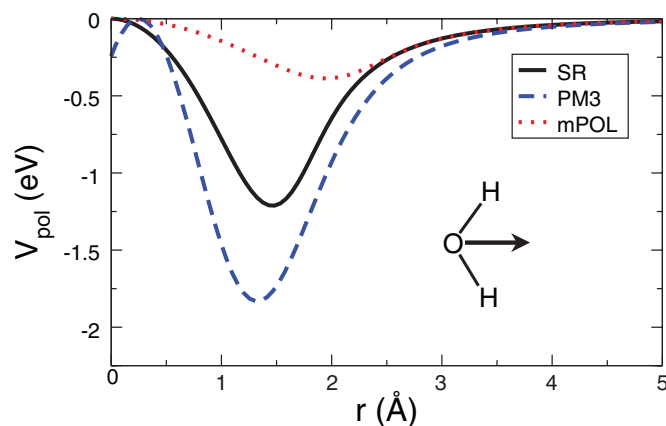
Madarász are correct in pointing out that our choice produces a ground-state eigenenergy for a single water molecule that is slightly overbound.

Despite this slight overbinding, figure 1 of our original paper (*1*) shows that our fit reproduces the features of the exact PK pseudopotential. Even though Turi and Madarász label the oscillations in our fitted potential ~ 4 Å away from the O atom as unphysical artifacts, these features are present in the exact numerical PK pseudopotential. Smoothing does shift the positions and amplitudes of these features slightly, but their presence likely has no effect in condensed-phase calculations, since an electron ~ 4 Å away from one water molecule in liquid water is certain to be much closer to, and hence interact more strongly with, the nearby water molecules.

Although the PK procedure generates a well-defined single-molecule pseudopotential, the properties of isolated gas-phase water molecules are not the same as those in bulk water (e.g., the dipole moment of a gas-phase water molecule is ~ 1.8 D, whereas in liquid water it is ~ 2.4 D), because interacting water molecules strongly polarize each other. We chose to incorporate the effects of this many-body polarization in two ways: We altered the point charges in our PK-based potential to match those of the classical water molecules (*8, 9*) used in our condensed-phase simulations, and we also grafted on a pairwise-additive polarization potential originally parameterized by Schnitker and Rossky (SR) (*10*). Although the SR polarization potential is ad hoc, Fig. 1 shows that it is remarkably similar to the more rigorously derived PM3 polarization potential (*11*). Thus, we expect our potential (*1*) to be physically reasonable within the not-inconsiderable constraints of a non-self-consistently determined polarization term, the assumption of pairwise additivity, the necessity of fitting to an analytic function, and the use of a classical water model parameterized for the bulk liquid.

Given how we developed our potential, we believe that Jacobson and Herbert's (*3*) claims of supposed errors in our development reflect a

Fig. 1. Comparison of electron-water pairwise-additive polarization potentials. The SR (*10*) polarization potential (black curve) used in our study is quite similar to Sommerfeld *et al.*'s (*11*) PM3 polarization potential (blue dashed curve), which corresponds to a Born-Oppenheimer limit of their Drude-oscillator configuration interaction model. For comparison, the modified polarization potential used by Jacobson and Herbert (*3*) (mPol) is shown as the red dotted curve. The mPol potential clearly underbinds electrons relative to the other polarization potentials that were designed for use in either cluster (PM3) or condensed-phase (SR) environments. The potentials are plotted as functions of distance, r , from the oxygen atom along the water dipole, as indicated by the inset.



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fundamental misunderstanding of pseudopotential theory. First, Jacobson and Herbert claim that our potential is overly attractive near the hydrogen atoms because the density of the water PK pseudo-orbital has a maximum in this region, whereas the density of the exact static-exchange wave function (HF LUMO) is at a minimum. However, this difference arises because the HF water LUMO has a node close to the H atoms, whereas the PK pseudo-orbital is nodeless by construction (4, 5). PK pseudopotentials are based on pseudo-orbitals, not LUMOs (4), and because the exact PK water pseudo-orbital has a density maximum near the H atoms [see, for example, figures 1 and 2 in (6)], this requires a minimum in the PK pseudopotential that reproduces this density. Second, Jacobson and Herbert note that our potential gives a different behavior from HF theory, from which it was derived. This is not surprising: As pointed out above, we modified the point charges in our PK potential and added the SR polarization term to account for (in a pairwise-additive way) the correlation/dispersion interactions that are missing in HF theory (11). Thus, optimizing a water cluster anion with HF theory and with our potential should lead to different structures with different wave functions.

Even though our potential was designed for the condensed phase, Jacobson and Herbert assert that cluster VEBEs are an appropriate benchmark for testing our potential. In their figure 1 (3), they compare VEBEs determined with our potential to MP2 calculations and conclude that our potential overbinds the electron in water anion clusters. Although we never expected our potential to provide an accurate means to replace high-level quantum chemistry calculations for water anion clusters, in

Table 1. VEBEs, in meV, of water cluster anions from ab initio calculations at the CCSD(T) level (11), our pseudopotential (LGS) (2), and the modification of the polarization term in our pseudopotential (LGS-mPol) proposed by Jacobson and Herbert (3); the number in parentheses shows the difference between the pairwise-additive and ab initio results. The mean differences from the ab initio results are for our LGS potential +40 meV (all clusters) and +38 meV (only $n = 6$ clusters), whereas those for Jacobson and Herbert's LGS-mPol potential are -165 meV (all clusters) and -197 meV (only $n = 6$ clusters).

Cluster* Ab initio†	LGS (diff)‡	LGS-mPol (diff)‡
W2 (S)	41	93 (+52)
W6A (S)	470	537 (+67)
W6B (S)	610	528 (-82)
W6C (S)	340	580 (+240)
W6D (S)	380	339 (-41)
W6E (I)	550	527 (-23)
W6F (I)	780	847 (+67)

*Cluster geometries are taken from (11). The number indicates the cluster size, the letter indicates which isomer, and the letter in parentheses denotes whether the electron is bound to the surface (S) or in the interior (I) of the cluster. †Calculated at the CCSD(T) level, taken from (11). ‡Calculated using identical grid parameters to the bulk e_{aq}^- simulations of (2).

Table 1 we show the energies of several small water anion clusters computed with both our potential and high-level coupled-cluster singles and doubles with perturbative triples [CCSD(T)] calculations (11). For all but one cluster (W6C), our potential reproduces the VEBEs remarkably well, a clear indication that it works in cases beyond that for which it was originally designed. It is worth noting that Jacobson and colleagues themselves have developed a (non-pairwise-additive) electron-water pseudopotential that accurately reproduces the VEBEs of water anion clusters (7); when they transferred this potential to the bulk, however, they got a noncavity electron, and this potential had to be substantially redesigned to remove the noncavity behavior (12).

We agree with Jacobson and Herbert that the apparent ~2 eV overbinding of the bulk electron with our potential could be of concern. However, given that condensed-phase VEBEs are sensitive to long-range interactions (13), we expect that one could make up the ~2 eV without inducing cavity formation by replacing our ad hoc polarization term with a many-body self-consistent polarization model [as was done by Herbert and Jacobson (12) to get a noncavity electron], so that the longer-range portion of the effective electron-water interaction would be weaker. This also would likely correct any discrepancy with the VEBEs of the larger clusters in Jacobson and Herbert's figure 1 (3).

Interestingly, both Turi and Madarász (2) and Jacobson and Herbert (3) propose modifications of our potential to correct for what they see as overbinding. In Turi and Madarász's case, our potential was adjusted to no longer have the correct physical form but to guarantee the correct single-molecule eigenenergy; in Jacobson and Herbert's case, the SR polarization term was effectively shut off (Fig. 1), leaving only the gas-phase PK pseudopotential, which underbinds the electron to small clusters (Table 1) but does better for larger clusters. What these modifications show is that potentials that are parameterized to match VEBEs for single molecules or small clusters can give extremely different behavior in the bulk. In fact, the potential in (6) is constrained to give the exact same single-molecule eigenenergy as that in figure 1B in (2), but these two potentials (which use distinct ad hoc polarization models) predict strikingly different bulk hydrated electrons. For example, Turi and Madarász's modification of our potential gives more than ten water molecules in the electron's first solvation shell, versus only four with the potential in (6).

What both of the critiques show is that fitting VEBEs does not constrain the universe of pseudopotentials sufficiently to define a unique pairwise-additive pseudopotential for use in the condensed phase. Neither Jacobson and Herbert (3) nor Turi and Madarász (2) make a case for why their chosen VEBE-based measure provides the best criterion by which to judge a potential, and there is no acknowledged "best" procedure in the literature for transferring single-molecule PK potentials for

use in condensed phases. We believe that when extending single-molecule potentials for use in condensed-phase calculations, it is most important to get the overall shape of the potential right if pairwise additivity is to have any hope of success. Thus, we contend that Turi and Madarász and Jacobson and Herbert's criticisms reflect these authors' biases about how to construct "good" potentials; their arguments provide grounds for legitimate scientific discourse, but they do not provide proof that any particular potential is the best for use in the bulk case.

In our original paper (1), we argued that the best way to test a potential for use in the bulk is to compare its predictions to condensed-phase experimental measurements. With the exception of the bulk VEBE discussed above, our hydrated electron simulations agree with the experimental absorption spectrum, polarized and unpolarized absorption transients, and diffusion constant. Jacobson and Herbert (3) claim that our potential gives a diffusion constant that is too large, but our simulations give $D = 2.0 \pm 0.6 \times 10^{-5} \text{ cm}^2/\text{s}$ (error bars are $\pm 2\sigma$), a factor of ~2 smaller than experiment (14) but similar to that given by most cavity models (15). Given that our simulations match so many experimental results as well or better than cavity-based models, we remain unconvinced that the VEBE criterion is all-important when determining the effectiveness of a potential for use in the condensed phase.

Turi and Madarász (2) have also pointed out that our results appear to contradict recent ab initio simulations (16), which show excess electrons in water occupying a cavity. Thus far, though, the simulations have been performed only for small numbers of water molecules in clusters. With our potential, we found substantial finite-size effects, such that even with 200 water molecules there were large fluctuations that did not occur with 500 waters (1). This sensitivity to system size could be indicative of the importance of long-range forces in establishing the solvation environment of the hydrated electron. Pending extension to larger system sizes and the further vetting of new density functionals (12, 17), we believe it is best to withhold judgment while noting our great interest in these ab initio results.

The current debate brings us to the question of precisely what is the proper balance between attraction and repulsion for electron-water interactions. It is well known (18) that for potentials dominated by repulsion, temperature changes have little effect on liquid/solute properties if the density is kept constant. In contrast, when attractive forces play a substantial role, changing the temperature at constant density alters solute properties. Experiments have shown that the absorption spectrum of the hydrated electron does shift with temperature at constant density (19), a result that is not reproduced by simulations based on a cavity model (20). Thus, we speculate that even if our potential proves to be somewhat too attractive, it will turn out that the balance between attraction

and repulsion also has not been properly accounted for in previous potentials.

We close by reiterating that although it was derived using a rigorous procedure, even if our potential were entirely ad hoc, it constitutes an existence proof that the known experimental properties of the hydrated electron are consistent with a noncavity picture. In addition to the discussion comparing properties of the noncavity electron to experiment in our paper (*I*), it is known experimentally that the molar solvation volume of the hydrated electron is negative (*2I*). This feature is consistent with the enhanced water density we see inside the noncavity electron with our potential (*I*) and is harder to rationalize with cavity-based models. Ultimately, as noted in our study, it will be up to future experiments and calculations to determine whether or not the hydrated electron resides in a cavity.

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CHEMISTRY

The Road to Flat TVs

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Over a 40-year professional involvement with liquid crystals—participating in conferences, meeting colleagues, making a few modest scientific contributions—I thought I had developed through osmosis a sense of the field's history. Reading David Dunmur and Tim Sluckin's *Soap, Science, and Flat-Screen TVs* revealed how fragmentary and incomplete a picture my approach has provided. Dunmur (a chemist) and Sluckin (a mathematical physicist) trace the chain of discoveries that has led to the most visible result of the 100-plus years' work in the field of liquid crystals: the flat-panel display now so ubiquitous in digital displays, computer screens, and 40-inch television screens.

The authors remain focused on this theme throughout the book. They highlight only those discoveries that, and those people who, made what can be considered fundamental contributions to the development of the materials, the theory, and the technology that have enabled flat-panel displays. Thus, readers expecting a comprehensive history of all aspects of the science and technology pertaining to liquid crystals may be disappointed. Nonetheless, the authors' delightful weaving of the influence of first individuals and then commercial companies with the advances in science pertinent to developing displays makes for fascinating and entertaining reading. Perhaps a quote from the authors' preface is appropriate here.

We do not have a fixed ideological view about how to present science to the non-expert, or about which type of historical explanation is valid and which is not. We are, after all, neither professional historians nor professional writers, but professional scientists....

... The reader will find a good deal of personal detail in our story, detail of how individuals came to their discoveries, what drove them, how they behaved when they were famous, and so on. For us, at least,

although it has explained little about the global development of the subject, it has provided a personal link with those who founded our subject and whose names decorate the phenomena with which we work every day.

I would disagree with the authors' concern that these details about individuals do not add to our understanding of the global nature of the field of liquid crystals. It can be argued that, in their tracing of the development of flat-panel displays, they definitely followed the national histories of the subject that flowed from one country to another along a time line and contributed to the whole. The field of liquid crystals is today unquestionably highly interdisciplinary and international.

The beginning of the science of liquid crystals is generally placed at Friedrich Reinitzer's observation in 1888 of two melting points of cholesterol benzoate, a substance isolated from carrots. When heated, the solid first melts (at 145.5°C) into a turbid liquid. Warmed another 33°C, this cloudy liquid turns clear. What is in a name, especially a name for a new phenomenon? Often plenty of argument, and so it was in the early days of liquid crystals. Liquid crystals, flowing crystals, mesophases, and the fourth state of matter were all terms applied to substances found to exhibit the properties of fluidity, turbidity, and a tem-

perature at which the "liquid" became completely clear. Of course, new advances have their champions and detractors—so too with liquid crystals. In the period between the discovery and the early 1930s, researchers debated the various names. Is liquid crystals the best name for substances exhibiting both properties of crystals (that is, order of the molecules comprising the material) and characteristics of liquids (flowing and not supporting a shear stress)? Probably mesophase, referring to a phase that exists between a crystal and a normal liquid, would be a better choice. It is the name preferred by professionals in the field today, but the public's eye is now fixed on liquid crystals.

After starting in Austria and Germany, the ebb and flow of the development of liquid crystalline materials, theory, and technology moved to France, expanded to the United Kingdom, and subsequently gained adherents in the United States, India, and Japan. Today, the centers of commercial development are found in companies in Japan and South Korea. This flow is described well by the authors. Dunmur and Sluckin provide an enticing and informative description of these shifts.

The development of flat-panel displays hinged on some critical experiments and the discovery of new mesomorphic materials. The crucial experiments demonstrated that the order that characterizes the molecular arrangements in a liquid crystalline phase could be disturbed by the application of magnetic or electric fields to the sample. This key observation is really at the heart of any liquid crystal display (LCD), and the authors carefully portray its history. The first substances found to exhibit liquid crystallinity generally had melting points well above room temperature and were usually sensitive to chemical decomposition. Because we want most display devices to operate at or near room tem-

Soap, Science, and Flat-Screen TVs
A History of Liquid Crystals

by David Dunmur
and Tim Sluckin

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An array of displays. LCD televisions at a consumer electronics trade fair.

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perature, suitable liquid crystalline materials had to be synthesized rather than simply derived from natural sources. How such materials were first discovered and then put to use forms a second major part of the story leading to LCDs.

One can argue that histories should provide a look to the future, and the authors have done just that. Their concluding chapter, "The new world of liquid crystal materials," offers a succinct but engaging answer to *quo vadis?* for liquid crystals.

I have consciously avoided filling this review with the names of the scientists and the details of their discoveries. That way, if you follow my recommendation to read *Soap, Science, and Flat-Screen TVs*, you can discover such details at your own leisure and pleasure.

10.1126/science.1203145

ENGINEERING

Living Inspired

Where does the inspiration for something new originate? For scientists and engineers, sometimes it appears in the cross-fertilization of known concepts from diverse fields or the rare flash of a new idea, but more often it comes from leveraging what is already known by building on things that work well. Increasingly, researchers are turning to nature for inspiration, by looking to organisms that do things we are unable to do on our own or do them better than we can—often functioning with an economical use of limited resources and energy. Animals that fly, explore deep under water, can see without light, or even stick to glass walls are all being studied with the hope of developing new materials, structures, or devices that may enhance our everyday lives.

Under the provocative and intriguing title of *Bulletproof Feathers*, Robert Allen (an engineer at the University of Southampton) brings together highlights from recent research in the broad areas of bioinspiration and biomimicry to explain, in simple terms, how physical scientists and engineers are increasingly using the study of the natural world as a starting point. Collectively, the six image-stuffed chapters (each written by a top researcher in the field) offer nonspecialists an intriguing sampler of bioinspiration. The various topics are organized around capabilities,

from looking at new material systems to challenges that span a range of animals, such as collaborative communication and the management of fluid flow and heat transfer.

Two chapters focus on animals that live in the sea: Jeanette Yen presents a range of potentially useful ideas drawn from marine biology, and Tomonari Akamatsu focuses on underwater bioacoustics. It is perhaps not surprising that so many recent discoveries have come from studying marine organisms. Our ability to watch and learn has been in part limited by the difficulties in building manned or remotely operated vehicles that allow us to study them under the challenging conditions underwater (especially at great depths). These very challenges are a good place to start when looking for inspiration. How do underwater creatures communicate when sound travels only limited distances, how do they see when sunlight only provides minimal lighting, and how do they avoid being crushed by the high hydrostatic pressures? As we find answers to these questions, we will gain insights for building improved vessels and equipment that can in turn teach us even more from the marine environment.

The chapter that differs the most from its companions is Yoseph Bar-Cohen's "Human-like robots." In this area, the principal goal isn't necessarily to learn from or copy nature but rather to design robots that are able to function in a world built around human capabilities. Tasks that even children are able to do with ease—such as crawling, walking, climbing, grabbing objects without dropping or crushing them, seeing obstacles, and hearing one's surroundings—can present substantial challenges when designing robotic devices.

Most industrial robots are designed to carry out repetitive, sometimes hazardous tasks. In carrying out such work, they have few direct interactions with humans, and thus there is no imperative to make them look like humans. In fact, humanlike characteristics can provide a downside because an operator might become emotionally attached to a machine assigned to dangerous jobs such as defusing explosives.

Accompanying the increasing interest in having robots interact with humans, there is a push toward making them more like humans. Practical applications include robots to aid the elderly (particularly in countries with an aging population) or to work in classroom settings (such as with autistic children). Lifelike robotics are also relevant in the designing of more-realistic prosthetics, and robots are even being used to study human behavior. For many of these purposes, we need to find ways to duplicate human efficiencies, such as our ability to generate energy from a range of fuel sources or our compact and dynamic muscles. Many of these challenges will require the development of new materials, although in some areas substantial progress has already been achieved (for example, the elastic polymer materials created by David Hanson that can be used to give robot heads lifelike facial expressions and gestures).

Biomimetic engineers have already learned a lot from nature, and no doubt the further study of organisms and their often surprising capabilities will suggest, for example, new ways to design materials, to create imaging and communication techniques, and to build stronger or more aerodynamic structures. The inspiration comes from observing what is normal for the organisms and wondering how they function and how we might mimic key features or superior properties.

Research often involves tedious laboratory work in isolated rooms or in large but remote user facilities—circumstances in which it may be easy to become uninspired. The rich collection of photographs in *Bulletproof Feathers* highlights state-of-the-art biomimetics research. Browsing through it, readers will encounter natural mechanisms that have stimulated researchers looking for new ideas. That may be as good a way as any to find your own inspirations and scientific connections.

—Marc Lavine

10.1126/science.1192323



Good for channeling light. The biosilica spines of this glass sponge, Venus flower basket (*Euplectella aspergillum*), share their design with those used in fiber-optic lines.

Bulletproof Feathers

How Science Uses Nature's Secrets to Design Cutting-Edge Technology

Robert Allen, Ed.

University of Chicago Press, Chicago, 2010. 192 pp. \$35, £22. ISBN 9780226014708.

An Emerging Role of Zoos to Conserve Biodiversity

D. A. Conde,^{1*} N. Flesness,² F. Colchero,¹ O. R. Jones,¹ A. Scheuerlein¹

Roughly one in seven threatened terrestrial vertebrate species are held in captivity, a resource for ex situ conservation efforts.

At the October 2010 meeting of the Convention on Biological Diversity (CBD) in Nagoya, Japan, delegates discussed a plan to reduce pressures on the planet's biodiversity. Key targets include expanding coverage of protected areas, halving the rate of loss of natural habitats, and preventing extinction of threatened species (1). For species whose habitat is severely threatened, however, the outlook is so bleak that the International Union for Conservation of Nature (IUCN), the U.S. Endangered Species Act, and the CBD (Article 9) recognize that in situ conservation actions (i.e., in the species' natural habitat) will need to be combined with ex situ approaches, such as captive breeding in zoos, aquariums, and so on (2, 3).

Captive breeding may be the only short-term practical conservation option for species confined to dwindling habitats (4). However, captive breeding is absent or plays a minor role in the policies of most governments, conservation organizations, and multilateral institutions. To shed light on the state of captive breeding and its potential to contribute to conservation goals, we estimate the number of threatened species already held in captivity.

Captive Breeding

Although ecosystem health should be a conservation priority, a recent evaluation of the status of the world's vertebrates (5) noted that captive breeding played a major role in the recovery of 17 of the 68 species whose threat level was reduced [e.g., Przewalski's wild horse (*Equus ferus przewalskii*) (6), black-footed ferret (*Mustela nigripes*) (7), and California condor (*Gymnogyps californianus*) (8)]. Captive breeding has the potential to maintain targeted populations as an "insurance policy" against threats like disease or pressure from nonnative species [e.g., egg predators on islands (9)] until reintroduction into the wild is possible. A striking example is the increase of amphibian collections in zoos (10) as a response to chytridiomycosis, a fungal infection responsible for precipitous

global amphibian population declines (11).

Captive breeding for reintroduction has downsides. Sociopolitical factors can determine the success of programs. For example, reintroduction of Arabian oryx (*Oryx leucoryx*) in central Oman was hampered by poaching, partly because local communities were insufficiently involved in conservation efforts (12, 13). Furthermore, captive breeding is costly, and technical difficulties can arise such as hybridization [breeding among different species (14), e.g., if current cryptic species are managed as one species, but are later split into several species according to new taxonomic information]. The ability of individuals to learn crucial skills that allow them to survive in the wild (e.g., fear of humans or predators) may be compromised. In many cases, these difficulties have been overcome by creative and species-specific measures. For example, it was feared that Puerto Rican parrots (*Amazona vittata*) would be unable to escape predators in the wild, but this problem was solved with a pre-release aviary-based stimulation and exercise program (15). Because ex situ conservation programs can be challenged when called into action at the last possible moment with only a few remaining individuals of a species, captive breeding should not simply be seen as "emergency-room treatment." It is a tool that should be considered before the species has reached the point of no return.

Counting Threatened Species in Captivity

We used the International Species Information System (ISIS) database to estimate the number of threatened species already held in captivity. ISIS is an organization that holds the most comprehensive information on animals held in zoos and aquariums worldwide, with records of ~2.6 million individuals shared among ~800 member institutions (16). From the IUCN Red List of Threatened Species (17), we obtained the threat category of each terrestrial vertebrate species represented in ISIS (18). [See supporting online materials (SOM) for details.]

One-quarter of the world's described bird species and almost 20% of the mammal species are held in ISIS zoos (table S1). Only 12% of the described reptile species are rep-

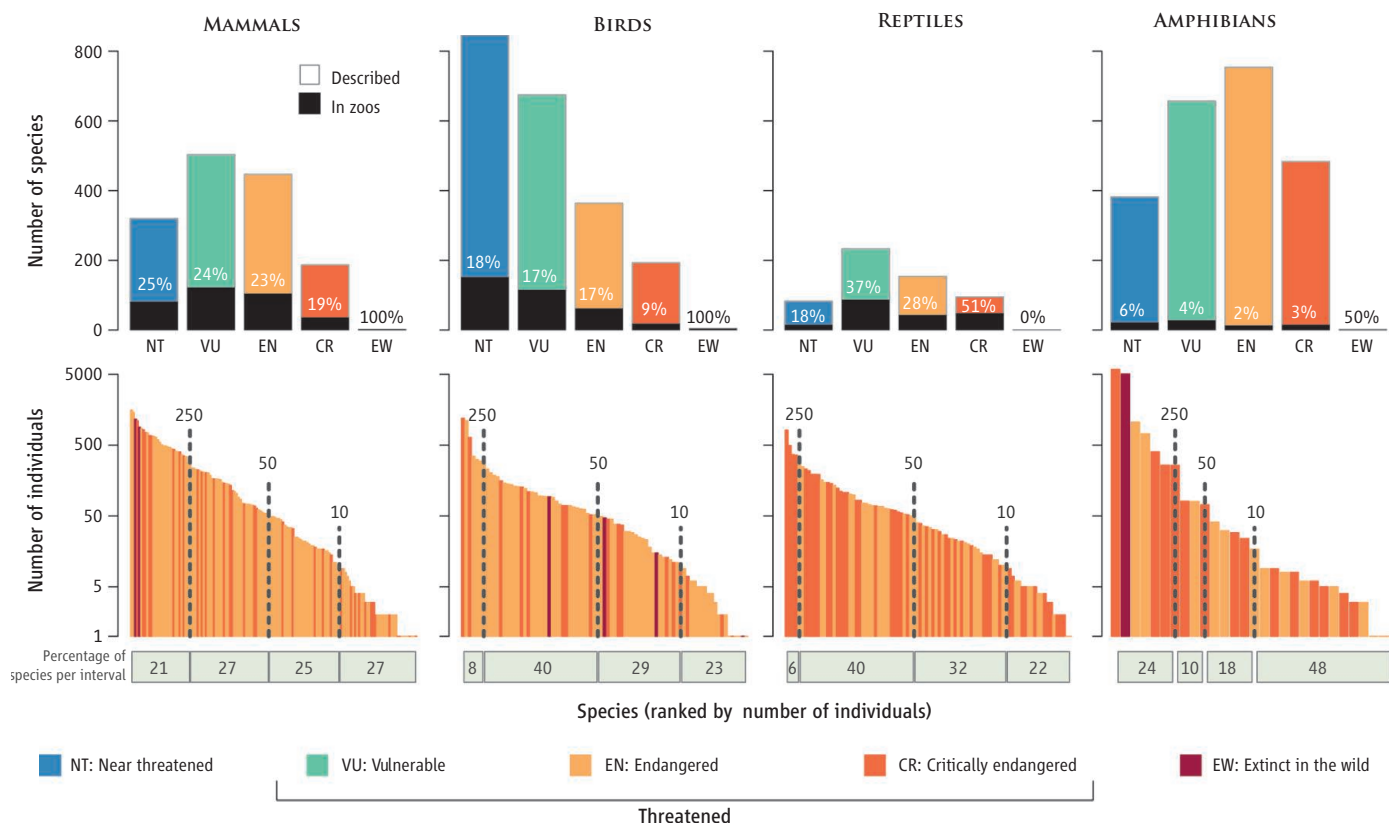
resented and 4% of amphibians. Our primary focus is on species of conservation concern; for mammals, roughly one-fifth to one-quarter of threatened (19) and Near-Threatened species are represented in ISIS zoos (see the figure) (table S1). With the exception of Critically Endangered species, which only have a 9% representation (tables S1 and S2), the picture is similar for birds. For amphibians, the representation of threatened species is much lower (~3%); this is a concern because amphibians are a highly threatened group, with 41% of described species listed as threatened or Extinct in the Wild (EW) (5). The IUCN threat-level assessment for reptiles has not been completed, so our results should be interpreted with caution, but of the 1672 species already evaluated, zoos hold 37% of threatened and 18% of Near-Threatened species.

Overall, zoos and aquariums hold roughly one in seven threatened species (15%), but it is important to consider also the number of individuals held. Although individual zoos might not have large populations of a particular species, collectively, zoos hold sizable populations of certain species, including highly threatened ones (see the figure). Zoos, as a global network, should strive to ensure that their populations of threatened species can survive in the long term. However, each zoo may make a larger conservation contribution by specializing in breeding a few at-risk targeted species, rather than aiming to increase its species diversity, as specialization increases breeding success (4).

Ultimately, success of conservation actions depends on the extent to which birth and death rates permit populations to survive in the wild (8). Population viability analyses (PVAs) are used to forecast the probability of population extinction for conservation programs (20), but these require parameterization with data on age-specific birth and death rates (21). Adequate data from natural environments are often unavailable, especially for threatened species (20). The zoo network has large long-term data sets, including data such as average litter size, interval between successive litters, and age at maturity, which could be used to fill these gaps. Of course, zoo data should be used with caution because they

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Endangered species in zoos. (Top) The number of species with IUCN status, globally described (color bars) and in ISIS zoos (black bars). **(Bottom)** The number of individuals in ISIS zoos for species listed by IUCN—for mammals (142 species), birds (83 species), reptiles (90 species), and amphibians (29 species). The vertical broken lines show the boundaries by 250, 50, and 10 individuals. The large numbers of individuals classified as Vulnerable and Near Threatened are omitted for clarity. See SOM for details.

do not necessarily reflect the situation in the wild, such as population flexibility in the face of changing conditions.

Despite their current and potential contributions to species conservation, ISIS zoos are concentrated in temperate regions, whereas most threatened species are tropical (5, 22) (fig. S1). This mismatch between the areas where captive populations are held and their native range poses a challenge for implementation of effective conservation actions. Acclimatization to a new home is likely to be faster for animals raised in conditions similar to those where they are to be released. This is one reason that it is suggested that captive breeding be done in the country of the species' origin (2).

There are large parts of the world with high biodiversity value, yet whose zoos are not well represented in a global network (fig. S1). Given the importance of having data avail-

able for design of conservation programs, policy-makers must encourage and facilitate the participation of zoos from regions with high levels of biodiversity threat in global networks, such as ISIS and the World Association of Zoos and Aquariums (WAZA).

The potential for zoos to contribute to conservation is not a new concept for the zoo community. Zoos and aquariums have developed conservation projects in the wild, alongside research and education programs (23). For example, members of WAZA collectively spend ~U.S. \$350 million per year on conservation actions in the wild, which makes them the third major contributor to conservation worldwide after the Nature Conservancy and the World Wildlife Fund global network (24). Given the scale of the biodiversity challenge, it is vital that conservation bodies and policy-makers consider the potential that zoos as a global network can provide.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/331/6023/1390/DC1

10.1126/science.1200674

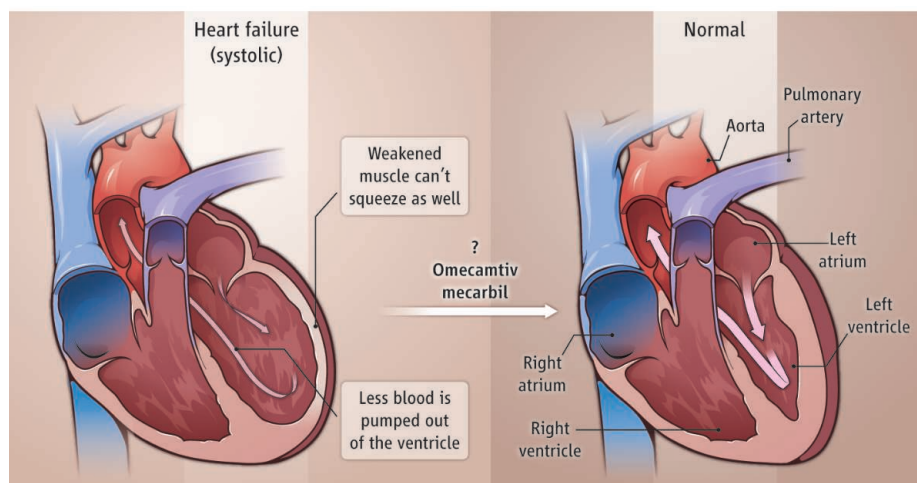
Chemically Tuned Myosin Motors

Leslie A. Leinwand¹ and Richard L. Moss²

Heart failure affects 5 million people a year in the United States, results in 300,000 deaths annually, and constitutes the single largest health care cost in the United States. Although some progress has been made in treating heart failure, currently approved drugs have deleterious consequences in some patients, and are ineffective in others. Therefore, new therapeutic approaches are necessary. On page 1439 of this issue, Malik *et al.* (1) elucidate the mechanism by which a small molecule enhances the function of cardiac myosin, the major force-generating motor protein of cardiac muscle. The finding has implications for improving cardiac contraction and treating certain heart conditions.

Cardiac myosin in humans and other mammals comprises isoforms α and β , which differ in their adenosine triphosphatase (ATPase) rates. Myocardial myosin binds to and hydrolyzes ATP. A cycle of this activity is associated with changes in myosin's conformation, its interaction with cytoskeletal actin filaments, and the generation of force, resulting in contraction of muscle fibers. In vitro studies of cardiac contractile proteins and intact cardiac myofibrils suggest that the speed of contraction is related to myosin isoform composition. Thus, ATPase activities, muscle shortening velocities, and rates of force development all increase as the proportion of the kinetically faster α isoform increases (2, 3).

In healthy human hearts, ~10% of myosin is expressed as the α isoform. However, this amount varies regionally in the heart's left ventricle, suggesting that its expression could be influenced by local variations in factors such as the amount of stress exerted on different regions of the ventricle's muscle wall during cardiac contraction. Human hearts in advanced stages of failure express no detectable α -myosin at all (4), and studies of healthy mice (5), rats (6), and pigs (7) predict that at least some of the contractile dysfunction observed in systolic heart failure—which results in failure to pump blood out of the heart and into the circulatory system (see the figure)—is due to reduced rates



Weak heart. In systolic heart failure, weakened contractions of the ventricle muscle wall pump less blood into circulation. Drugs that increase cardiac muscle contraction may be therapeutically useful.

of force development and myocardial work as a consequence of reduced cardiac α -myosin expression. Indeed, heart failure patients who respond positively to existing therapies show improved contractile function and reexpress α -myosin, whereas nonresponding patients do not (8). These results (and others) indicate that contractile function can be modulated by the ATPase activity of myosin and that targeting this activity could be used to treat systolic heart failure.

The discovery by Malik *et al.* that the small molecule omecamtiv mecarbil increases the efficiency of heart muscle contraction supports this idea. Omecamtiv mecarbil increases the ATPase activity of mammalian cardiac myosin in solution, the contractile function of cardiac myocytes (isolated from adult rat), and cardiac contractility (in both adult rat and dog) by binding to cardiac myosins. The main advantage of this approach over existing therapies is the specificity of this small molecule for a subset of myosins that are only expressed in a limited set of tissues. Therefore, compared with existing therapies, this small molecule would not be expected to affect organs that do not express these myosins. According to the amino acid sequence of the region in myosin targeted by omecamtiv mecarbil, the drug should only affect the function of four mammalian striated muscle (cardiac and skeletal) myosins (α , β , 7b, and 15), but not smooth muscle or other nonmuscle myosins. α -Myosin is expressed exclusively, and in a

A molecule that activates the contractile protein myosin points to a potential new means of treating heart failure.

low (though functionally important) amount, in the healthy human heart, and myosins 7b and 15 are expressed at even lower amounts in cardiac and skeletal muscles. As a result of these expression patterns, β -myosin, the major isoform in the adult human heart, would essentially be the only myosin targeted by omecamtiv mecarbil in heart failure. Importantly, the fast isoforms of human skeletal myosins (myosin IIa and IIb) are not affected by this molecule. However, the compound would affect the function of the β -myosin that is expressed abundantly in slow skeletal muscle fibers. These slow-contracting skeletal muscles would be expected to exhibit stronger, more efficient contractions in the presence of omecamtiv mecarbil.

Malik *et al.* show that omecamtiv mecarbil increases the rate of ATP turnover by accelerating actin-dependent phosphate release, which is the rate-limiting step in the actin-myosin ATPase cycle in contracting muscle (9), and also by slowing the rate of adenosine 5'-diphosphate (ADP) release. Together, these effects should increase the number of myosins that bind to actin in a force-producing state, thereby increasing force output and improving contractile function. Indeed, this was observed when the small molecule was administered to healthy dogs and to dogs in which an experimental form of systolic heart failure was induced.

Malik *et al.* also eliminated several possible side-effects of omecamtiv mecarbil that could limit its therapeutic usefulness,

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especially the concern that it could increase the energetic demands of maintaining calcium homeostasis during the cardiac cycle. The drug also did not affect the metabolic demands of myocardium in the adult rat, whether healthy or failing, which is a considerable advantage over existing treatments for systolic heart failure that change the contractile force of myocardial fibers.

Previous studies in animals and humans suggested that cardiac function could be improved by increasing the expression of fast α -myosin to accelerate the rate of force development. Omecamtiv mecarbil, by contrast, modifies the transition rate of β -myosin to the actin-bound force-producing state. This mechanism of action could potentially

increase the rate of force development without increasing the metabolic demands of a faster cycling myosin. The combination of increased phosphate release and slowed ADP release due to omecamtiv mecarbil increases the rate of force development, but the increase in force is also due to a longer occupancy time of myosin on actin. Thus, stress exerted in the heart wall muscle is increased with minimal increases in energy utilization, which is advantageous in cells that may be severely metabolically compromised in advanced stages of systolic heart failure. Based on the effects of other therapeutic interventions, treatment with omecamtiv mecarbil might also result in restoration of expression of the α -myosin isoform toward levels observed in

healthy individuals, thereby further improving systolic function.

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PLANETARY SCIENCE

Precipitation Climatology on Titan

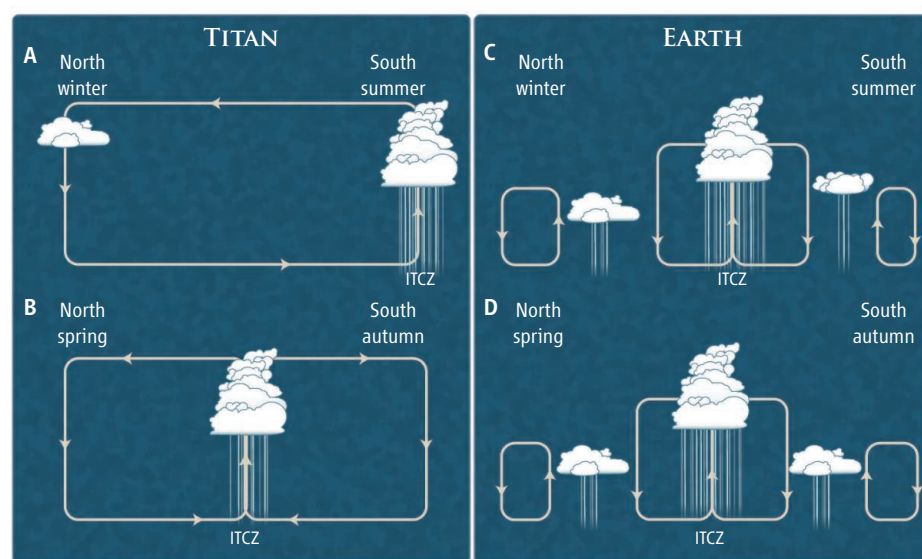
Tetsuya Tokano

The climate of planets is often described in terms of surface temperature only. In contrast, the widely used climate classification of Köppen (1) considers not only the surface temperature but also precipitation. Saturn's moon Titan is a rare extraterrestrial body where a similar concept could be applied to describe the climate because there is a methane hydrological cycle with rivers, methane vapor, clouds, and rainfall. But information on Titan's rain has been scarce. Although bright methane clouds can be monitored from Earth-based telescopes, rainfall is more difficult to observe from space, and there are no rain gauges or weather radars on the surface. However, precipitation can change the surface appearance in ways that can be observed by the Cassini spacecraft. Just as countrywide brightening of Earth's surface seen on weather satellite images can be interpreted as recent snowfall if a similar-sized cloud system was observed beforehand, similar ideas can be applied to Titan to monitor rainfall. On page 1414 of this issue, Turtle *et al.* (2) report the first observational evidence of substantial rainfall in the equatorial region of Titan. The appearance of a large cloud system was soon followed by surface darkening of an area east of this cloud, most likely by methane rainfall.

Titan's equatorial region has dry rivers, which indicate rainfall in the past (3), but it was unknown when the last rainfall occurred that filled such rivers; only observational evidence of weak drizzle (4, 5) had been obtained. The new observations by Turtle *et al.* add an important piece to this puzzle. The timing of rainfall is the crucial constraint. Rain predictions by numerical models diverge, but the model of Mitchell *et al.* (6) predicted substan-

Recent equatorial rainfall on Titan reveals the global-scale seasonal migration of its intertropical convergence zone.

tial tropical rainfall after equinox, in agreement with the present observation. According to this model, tropical rainfall occurs in this season because the intertropical convergence zone (ITCZ)—the region where the surface winds from the northern and southern hemispheres converge—passes over the equator. Seasonal swing of the ITCZ by itself is no exotic phenomenon. But why are there apparently long dry seasons at Titan's equa-



Cloudy with rain. Simplified global atmospheric circulation and precipitation patterns on Titan and Earth. Most precipitation occurs at the ITCZ, where air ascends as a result of convergence of surface winds from the northern and southern directions. Titan's ITCZ was previously near the south pole (A) but is currently on its way to the north pole (B). The seasonal migration of the ITCZ on Earth is much smaller (C and D).

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tor, in contrast to Earth's tropical rainforest climate? Earth's ITCZ can migrate only within the tropics because the large latitudinal temperature contrast at mid-latitudes gives rise to mid-latitude weather systems, which prevent the ITCZ from shifting further poleward. Because the tropical rain belt is often wider than its seasonal excursion, the equator can barely escape rainfall in any season. Conversely, Titan's ITCZ is predicted to migrate nearly from pole to pole, so that it rarely stays near the equator (6). This dramatic global migration of the ITCZ is a consequence of Titan's slow planetary rotation (15 days, 22 hours), which requires the ITCZ to extend far beyond Titan's tropic (26.7°) and proceed to the summer pole to ensure conservation of angular momentum and energy (7). This pattern reverses twice in the course of an orbital cycle of Saturn (29.5 years). During recent years, the ITCZ of Titan was presumably located near the south pole and is now on the way to the north pole.

Although Titan's precipitation pattern is generally explained by global atmospheric circulation, it is also thought to be highly sensitive to several uncertain factors such as surface moisture (6), tropospheric ethane abundance (8), or chemical composition of polar lakes (9). On regional scales, orography (terrain) may also affect the rain distribution substantially (10). The lack of knowledge of many of these parameters, and the difference in physicochemical behavior between water and methane, thus makes rain prediction a challenging task. An individual rainstorm on Titan could cause surface precipitation of a few hundred millimeters within hours (11, 12), but the question is how often such rainstorms occur at individual places.

Titan's precipitation climatology is clearly different from that of Earth, and exotic climate zones unknown in Köppen's classification (1) may exist. Observations such as those by Turtle *et al.* reveal the geographical distribution, seasonal timing, interval

between rainstorms, and relative intensity of rainstorms, although smaller-scale or weaker rainfall might escape detection. It will also be helpful to search for changes in cloud top altitude (12), rivers, or polar lakes, as they may be diagnostic of surface precipitation.

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NEUROSCIENCE

A CRY to Rise

Seol Hee Im and Paul H. Taghert

When we need to wake at a certain time, some of us resort to setting several alarm clocks to ring at different times, or in different locations, to ensure that we don't oversleep. The biological timing system appears to use a similar strategy: It relies on a multi-tiered approach to detect environmental signals and deliver multifaceted information regarding ambient light conditions. In *Drosophila*, a light-sensitive protein called CRYPTOCHROME (CRY) serves as a light sensor that each day helps to reset the fly's circadian clock. On page 1409 of this issue, Fogle *et al.* (1) show that CRY also directly increases the firing rate of critical circadian "pacemaker" neurons by sensing blue wavelengths of light. This represents a mechanism for photoactivation of neurons that is not based on opsin, the protein typically involved in light-responsive brain activity.

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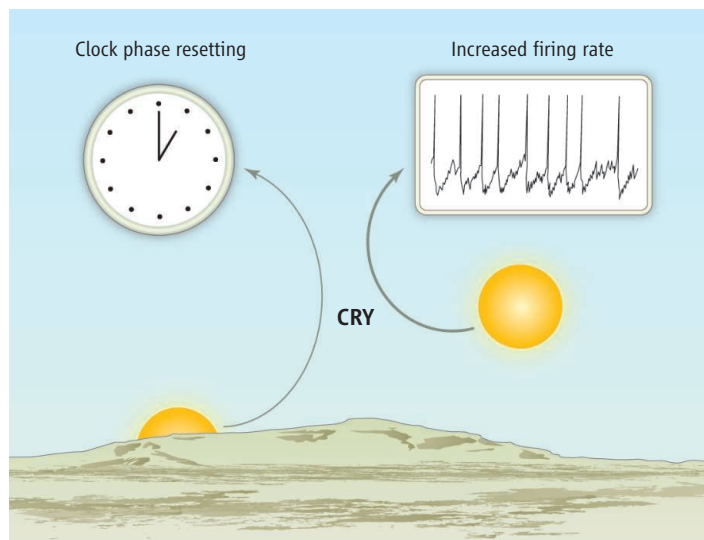
In *Drosophila*, the *cry* gene was first identified in experiments in which a *PER::luciferase* transgene (*per-luc*) generated bioluminescent pulses that researchers used to monitor molecular circadian rhythms and identify mutations that abolished the luciferase rhythms (2). Flies carrying the *cry* mutant *cry-baby* exhibit normal rhythmic behavior under light:dark cycles and constant darkness, but the mutants are less

Light-sensing CRYPTOCHROME activates the firing of neurons involved in circadian rhythms.

responsive to changes in the lighting schedule that would normally change the phase of subsequent behavioral cycles (2). CRY resets the fly's circadian clock by controlling the levels of a key circadian protein, TIMELESS (TIM). Upon light activation, CRY interacts with TIM, which promotes TIM's interaction with the F-box protein JETLAG and its subsequent degradation (3, 4). Over the course of hours, TIM degradation results in a resetting of the clock's phase. With prolonged light exposure, CRY itself undergoes degradation.

Fogle *et al.* use intracellular recordings from the cell bodies of specific circadian pacemaker neurons called the large lateral ventral neurons (ILNVs) to reveal a much faster form of

Seeing the light. Very low light levels at dawn and dusk (left) reset the circadian clock in neuronal pacemakers by changing levels of the TIM protein. Higher light levels at mid-morning (right) modulate electrical activity in the same neurons without TIM involvement. The multifunctional CRY protein mediates both aspects of this neuronal photosensitivity.



CRY-dependent light detection. There are ~150 neurons in the fly brain that express large amounts of the proteins involved in the circadian clock mechanism. Collectively, these 150 neurons act as a pacemaker network to control daily rhythmic behavior (5). Within that network, the eight ILNv pacemakers appear to have dedicated roles as light detectors to modulate arousal states (6–8); their levels of activation predict the fly's subsequent levels of behavioral activity. Fogle *et al.* show that light controls the rate of action potential generation (the “firing” rate) of the ILNvs and then argue that this is a cell-autonomous (i.e., cell-intrinsic) response by ILNvs to light. Their most compelling experiments show that CRY expression is not only necessary but also sufficient: Misexpression of CRY by nonresponsive neurons also causes them to respond to light.

This CRY-mediated electrophysiological light response in ILNvs is TIM-independent: Similar changes were observed even in flies carrying a *timeless* mutant. This suggests that within circadian pacemaker neurons, CRY has two distinct means of signaling light information (see the figure). This possibility was strengthened by experiments that compared the minimum light levels needed for each action. Clock resetting by CRY occurs at vanishingly low light levels (9). In contrast, increases in pacemaker firing rate required ~5 times the intensity necessary to induce CRY-TIM interaction. These higher levels correspond to natural light levels that are typically observed in early to midmorning.

These results are also striking because they reinforce several observations suggesting that the CRY family is remarkably multifunctional and diverse. As first shown in plants, CRY not only is a photosensor, but also is sensitive to magnetic fields (a magnetosensor) (10, 11). In mammals, the paralogous CRY1 and CRY2 proteins are also critical circadian elements, but not as photosensors. Instead, they form dimers with PERIOD proteins and can act as transcriptional repressors. Furthermore, in the retina, CRY mediates (directly or indirectly) the pupillary light response (12). Even among insects, the roles of CRY proteins vary. For example, butterflies have two separate *cry* genes: Butterfly CRY1 is a photosensor with no transcriptional activity, and butterfly CRY2 exhibits the functionality of the mammalian CRYs [i.e., with little or no photosensitivity (13)]. Fogle *et al.* show clearly that among insect CRYs, it is the photosensitive forms, and not the transcriptionally effective forms, that promote the direct increase in ILNv firing rate.

The autonomous responsiveness of

ILNvs to light is especially intriguing because it presents a striking evolutionary parallel. The response closely resembles that of intrinsically photosensitive retinal ganglion cells (ipRGCs) in mammals that express melanopsin (14). ipRGCs project to several brain regions (including the suprachiasmatic nucleus and the olivary pretectal nucleus) involved in circadian clock entrainment and radiance detection. Like the ipRGCs, ILNvs respond to light with increased action potential firing rates and not, as seen in other photosensitive sensory cells, with graded responses. Recent information indicates that ipRGCs indeed contribute to image-forming (15). The degree to which ILNvs may also contribute remains to be tested.

Xiang *et al.* (16) recently reported that *Drosophila* larvae are able to detect light via intrinsically light-sensitive peripheral neurons that tile the body wall. Apparently, these peripheral neurons (which help to drive light-avoidance behavior at high light intensities) and adult pacemaker neurons are similar, in that light responses act through novel transduction pathways in both cases. The

field must now address the questions of why so much photosensitivity is distributed so broadly, across the brain and throughout the body, and whether this capacity also extends to larger-bodied animals.

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CHEMISTRY

Heat and Light Switch a Chiral Catalyst and Its Products

Takashi Ooi

A rotary molecular motor acts as a chiral catalyst and can produce products of either handedness after external stimuli change its structure.

A right-handed glove won't fit a left hand because our hands are chiral. They are mirror images of each other, but despite this symmetry, their shapes cannot be superimposed one on the other. Molecules that are nonidentical mirror images are called enantiomers. Most biomolecules are found only as one of the two enantiomeric forms, and drug molecules that interact with biomolecular targets are usually more active in pure enantiomeric form. Chiral molecules can be prepared from the readily available, achiral starting materials, and many reactions that favor one chiral product (asymmetric reactions) do so by using a catalyst that itself is chiral (1). However, a versatile method must be able to make both left- and right-handed

products, which usually requires having to make both enantiomers of the catalyst. On page 1429 of this issue, Wang and Feringa (2) introduce a conceptually new approach in which the structure of a single catalyst can be manipulated by cycles of light and heat to pulses. These processes drive internal rotation around a carbon-carbon double bond and create two forms of the catalysts that give reaction products of opposite chirality (3).

Many of the catalysts used for asymmetric synthesis consist of a transition metal and a chiral molecule, called a ligand, that binds tightly to the metal center (4). The choice of metal and the structure of the ligand are both critical. The use of the two different metals in combination with the same enantiomer of a ligand sometimes allows for the selective production of either product enantiomer in certain reactions (5). Chiral organocatalysts,

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which are enantiopure, chiral small organic molecules, have recently emerged as a powerful alternative approach (6). Nagasawa and co-workers reported the synthesis of either enantiomer of a chiral product by changing the solvent and reaction temperature of a flexible organic catalyst (7).

However, it is very difficult to switch the handedness of the product produced by a single-enantiomer catalyst once it is in solution (8). Wang and Feringa pursued a completely different strategy. They prepared an enantiopure chiral alkene (**1**) (see the figure) with similar structural components on opposite sides of a double bond (the trans geometry; cis denotes the groups being on the same side). The two phenyl-substituted indane groups closest to the double bond are chiral, so they also create a helical orientation about the double bond (*P* and *M* denote right- and left-handed helicities, respectively). In addition, two functional groups often used in organocatalysis, thiourea (9) and dimethylaminopyridine (DMAP) (10),

are attached to the terminal of each phenyl-indane component (orange and blue panels in the figure) (11).

Like other spatially crowded chiral alkenes, molecule **1** is a molecular motor that converts the energy provided by light and heat into repetitive one-way rotation around the central double bond. Starting from the most stable (*P,P*)-*trans*-**1** configuration, the upper moiety of the molecule does one full clockwise rotation in four individual steps. Two are light-induced cis-trans isomerization associated with 180° rotation around the double bond, and the other two are inversion of the helicity controlled by heat (switching *P* to *M*). Because the terminal acidic (thiourea) and basic (DMAP) functionalities can only be in close proximity when cis isomers are generated during the rotation, Wang and Feringa envisioned that this rotary molecular motor could control asymmetric organocatalysis.

Wang and Feringa apply their system to the carbon-sulfur bond-forming reac-

tion between thiophenol (red in the figure) and cyclohexenone (blue in the figure) (12). Cooperative effects of the two catalytic functionalities on the molecular motor can activate both reactants for bond formation. Attempts using each pre-prepared stereoisomer of **1** as a catalyst showed that only (*P,P*)-*cis*-**1** and (*M,M*)-*cis*-**1** exhibited sufficient catalytic performance, whereas (*P,P*)-*trans*-**1** barely promoted the reaction. A different enantiomer was produced in excess (75% of one enantiomer and 25% of the other) in these two catalyzed reactions. The small amount of product obtained with (*P,P*)-*trans*-**1** was a mixture of an equal amount of enantiomers.

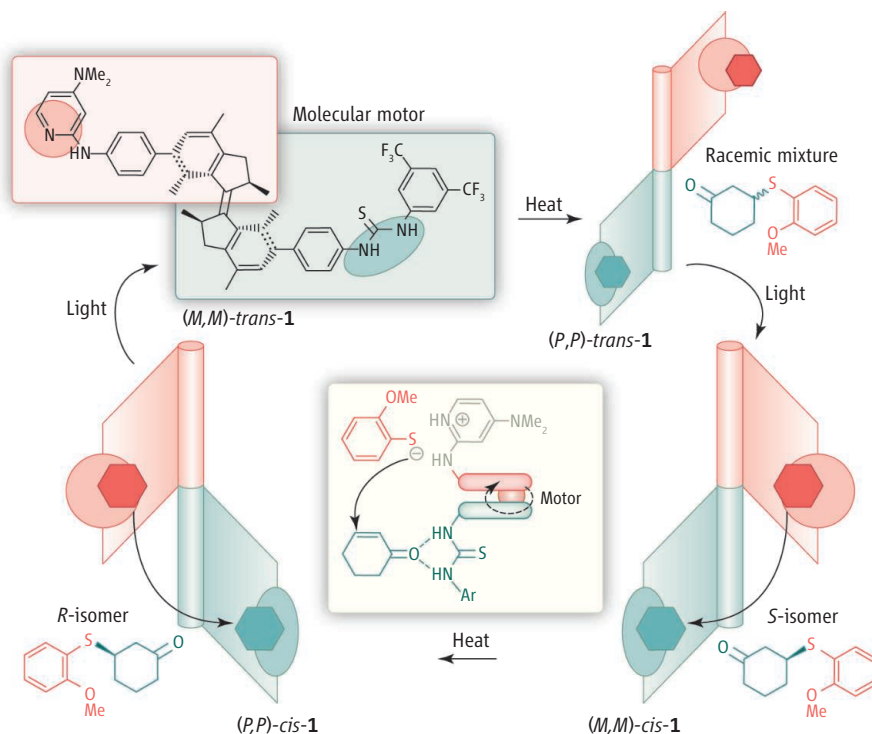
Control of the stereochemistry of the product was also demonstrated by monitoring the dynamic behavior of the inactive (*P,P*)-*trans*-**1** catalyst in a solution containing the two reactants. Ultraviolet irradiation caused rapid photochemical isomerization to the active (*M,M*)-*cis*-**1**, whereupon the reaction started to proceed smoothly. Additional computational analysis of the actual bond-forming stage of the reaction provides a convincing rationale for the formation of different enantiomers depending on the helicity of the cis isomers.

The work of Wang and Feringa offers unprecedented possibilities for the design of cooperative asymmetric catalysis. An important goal would be the construction of dynamic systems from a single molecular framework that can sequentially adopt defined numbers of stereoisomers in response to light or heat. Such catalysts could perform consecutive steps in the synthesis of chiral organic molecules in a single pot. Efforts in this direction may lead to the development of broadly useful catalytic machines driven by readily available physical stimuli.

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Turning out different products. Two components of the rotary molecular motor [see upper left, (*M,M*)-*trans*-**1**] of Wang and Feringa are represented by blue and orange panels. The active functional groups are thiourea (blue circle) in one component and dimethylaminopyridine (red circle) in the other. These groups can activate cyclohexenone (blue hexagon) and thiophenol (red hexagon), respectively, as shown in the center. With the most stable (*P,P*)-*trans*-**1** (upper right) as a catalyst, the active functional groups (blue and red circles) are far apart, and only a small amount of racemic product forms. Upon irradiation, the orange panel rotates to afford (*M,M*)-*cis*-**1**, which allows the active functional groups to interact cooperatively. This form is not only much more reactive but also creates an excess of the *S* enantiomer. Further isomerization to (*P,P*)-*cis*-**1** by heat changes the helicity of the catalyst so that the *R* enantiomer is obtained with similar efficiency. This stereoisomer can be converted back to (*P,P*)-*trans*-**1** by light and heat through the unstable (*M,M*)-*trans*-**1**.

GEOCHEMISTRY

Meteoritic Clues Point Chromium Toward Earth's Core

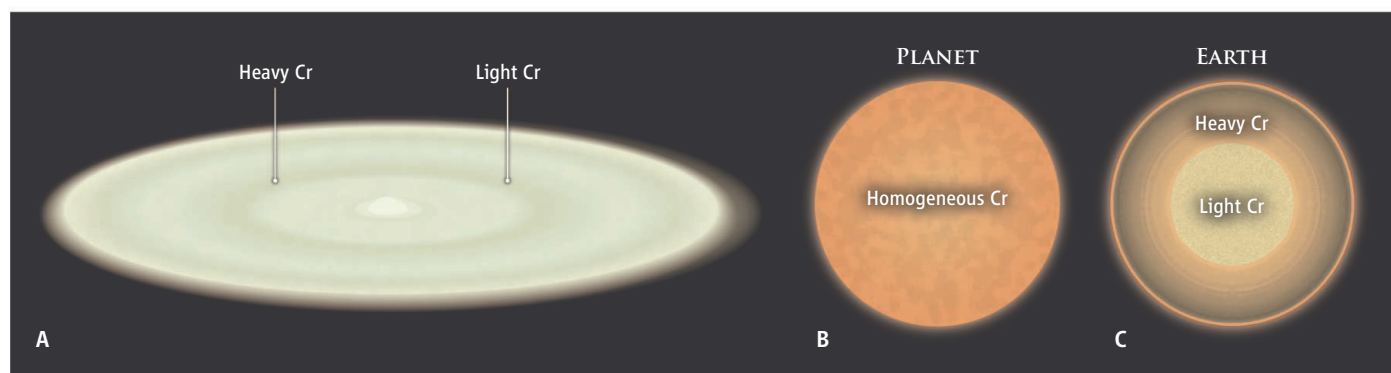
William F. McDonough

Chromium, named for the Greek word for color, is a wonderful element that adds green to emeralds, red to rubies, brilliance to plated metals, and corrosion-proof quality to stainless steels. It is Earth's 10th most abundant element and is distributed almost equally between the metallic core and the rest of the planet. It normally occurs as a trivalent ion (able to form bonds with three other atoms), but there is also an oxidized hexavalent form that is hazardous to life. By studying the distribution of chromium's isotopic forms on Earth and in the solar system, researchers can gain insight into the processes that formed planets and

processing, planetary accretion, and core-mantle separation. Moynier *et al.* reveal that the accessible parts of Earth (the mantle and crust, but not the core) have a chromium isotopic composition that is distinct from that of chondritic meteorites, the primitive, undifferentiated building blocks of the solar system. They assume that Earth's initial planetary composition was similar to these meteorites and conclude that there are now two distinct but complementary compositional domains inside Earth: the accessible portions that we analyze, and the inaccessible core. Moynier *et al.* conclude that this compositional difference is due to a process known as "core

The different chromium isotopic compositions of chondrites and the mantle offer insight into Earth's early history.

explore how material is exchanged between the core and the mantle (5, 6). Core formation chemically fractionated, or redistributed, stable isotopes of silicon and chromium. Tungsten isotopes record the fractionation of hafnium (Hf), which stayed in the silicate Earth, and tungsten, 90% of which was sequestered into the core. The short-lived, now-extinct isotope ^{182}Hf decayed to ^{182}W during the first ~60 million years of Earth history. Due to early core-mantle separation, excess ^{182}W remained in the silicate Earth, leaving it enriched in this isotope compared with primitive meteorites. Collectively, these differences, plus osmium isotopic abun-



produced the differentiation of Earth's core and mantle. On page 1417 of this issue, Moynier *et al.* (1) report that Earth's chromium isotopic composition is distinct from most primitive meteorites, lending support to one explanation for core and mantle formation. They also report that chromium's isotopic composition varies among different groups of meteorites, suggesting that the early solar nebula had at least two components with different isotopic compositions.

The isotopic compositions of materials provide DNA-like fingerprints that researchers can use to identify inorganic objects with shared histories or origins. In chromium, the relative abundance of its four isotopes—50, 52, 53, and 54—provides clues to the element's chemical behavior during nebula

partitioning," rather than an alternative process involving the volatilization of certain chromium isotopes. Core partitioning took place during Earth's early history at high temperatures, when the core separated from the silicate earth, leaving the core with a distinct composition that is enriched with lighter chromium isotopes (see the figure).

The excitement for the earth sciences community is that this finding adds another investigative tool for understanding and documenting past and present planetary processes. For the cosmochemistry and meteoritics communities, the findings further bolster the view that the solar nebula was a heterogeneous mixture of different components.

Over the past decade, studies have found that the isotopic composition of tungsten (W) (2, 3), silicon (4), and now chromium in chondritic meteorites differs from that of Earth's silicate shell (the mantle and the crust). These differences, in turn, provide additional means by which investigators can

Chromium's origins. New evidence suggests that, in the early solar nebula (A), chromium isotopes were divided into two components, one containing light isotopes, the other heavy isotopes. In the early Earth (B), these components formed a homogeneous mixture. During core partitioning (C), the core became enriched with lighter chromium isotopes, and the mantle with heavier isotopes.

dances (6) (which is inferred to vary between the inner and outer core), can be used to test the hypothesis that there is core-mantle exchange across a leaking boundary layer.

For instance, magmas at intraplate centers, such as Hawaii, are believed to originate at the core-mantle boundary. However, studies of osmium (6) and tungsten (5) isotopes from Hawaiian lavas, inferred to carry signatures of such mass exchange, have not resolved the issue (7). This equivocal state exists because researchers have had to make assumptions about the relative abundances of the elements in the core and mantle, and about the differ-

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ences in the isotopic composition of the two domains, and because of the precision of the measurements needed to resolve these issues. Now, investigations of core-mantle exchange can be extended using chromium isotopes.

A related question—What is the chromium isotopic composition of the Moon?—can also be explored. Lunar rocks have oxygen and tungsten isotopic compositions that are identical to that of Earth's mantle and, thus, Earth's distinctive chromium isotopic composition might also be found in lunar rocks. If not, would this imply that chromium isotopes were fractionated during lunar core formation or during the giant impact that is believed to have created the Moon?

Another stimulating issue arising from Moynier *et al.* is what chromium isotopes tell us about processes in the solar nebula and chondrite formation, which represents the least understood first step in planet formation (8). It appears that different types of chondrites, and even subgroups within these types, have different isotopic compositions. Major classes of chondrites can

be distinguished by the oxidation state of their metal-loving elements. These may be dominant in the reduced, iron-nickel (Fe-Ni) alloy form (e.g., as in enstatite chondrites); in the oxidized forms, where iron almost always occurs as an oxide (e.g., as in most carbonaceous chondrites); or as a mixture of both (e.g., as in common ordinary chondrites, which represent ~90% of all chondrites). Moynier *et al.* show that the ordinary chondrites generally have heavier chromium isotopic compositions than carbonaceous chondrites. At the same time, they show that those subgroups of carbonaceous chondrites with greater amounts of refractory materials (known as the CO and CV types) also have increasingly heavier chromium isotopic compositions. The issue for cosmochemists and astrophysicists is how this information can be used to improve models of mixing in the solar nebula and chondrite formation.

For more than half a century, it has been observed that meteorites (9) and planets (10) are composed of both a refractory (and per-

haps reduced) component and a volatile-rich, oxidized component. It is hoped that a certain brilliance will come from plating this story with chromium isotopes, which can contribute to unraveling where, in time and space, these components existed and were mixed together.

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CHEMISTRY

Tracking State-to-State Bimolecular Reaction Dynamics in Solution

Stephen Bradforth

If two reactant molecules collide with sufficient energy and the geometry between them is right, then the reactants can pass through a transition state to form new chemical products. A “state-to-state” study maps out how distributing energy (such as in different bond vibrations) in the reactant states affects the likelihood of reaction, as well as the corresponding state population of the products. For gas-phase reactions, the product states, rather than being populated statistically (with population falling off exponentially with increasing energy), can instead show higher population in excited states than the ground state. This so-called state inversion is the basis for the powerful HF chemical laser (1). But does this state-to-state behavior matter at all for bimolecular reactions run in liquids? The constant fluctuations of the solvent should randomize the state of the reac-

tant right up to the transition state, and speed the relaxation of product excited states back to a statistical distribution. On page 1423 of this issue, Greaves *et al.* (2) show that a vibrational state inversion can in fact be achieved in a hydrogen transfer reaction in a common liquid solvent by tuning the time scales of the relaxation processes.

In the 1960s, Polanyi formulated a simple set of rules for what energy states in the reactants would enhance reaction, and in what states the energy released should appear (3). These rules, for example, explained why the reaction powering the HF chemical laser, the transfer of a hydrogen atom between molecular hydrogen and fluorine to produce hydrogen fluoride, exhibited a vibrational state inversion (1). Modern gas-phase reaction dynamics, aided by laser-assisted reactant-state preparation and product-state interrogation, now provides stunning detail on the bimolecular reactions of small molecules, revealing the detailed forces acting at the transition state (4, 5). Studies under vacuum

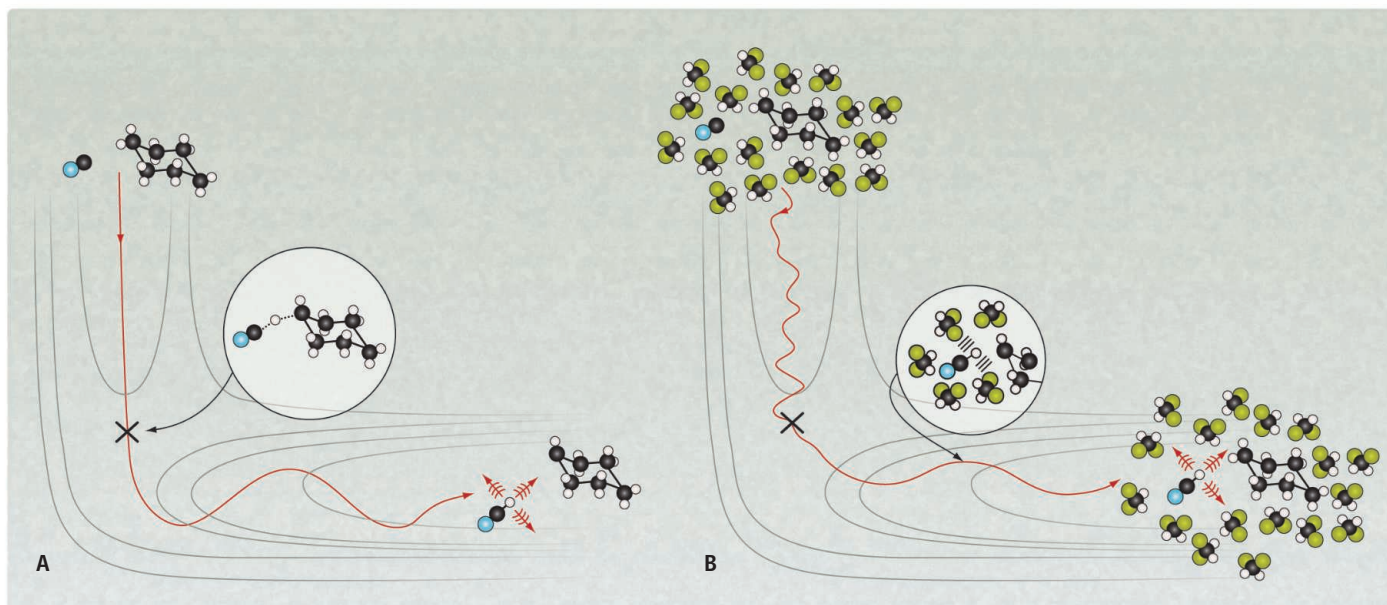
A bimolecular reaction can produce a large fraction of highly vibrationally excited products despite the collision and confinement effects of the surrounding liquid.

conditions can ensure that the only collision between two molecules is the reactive event itself, and that all of the information encoded in the product state distribution is preserved.

In liquids, it is hard to observe such nonstatistical product-state distributions precisely because the constant buffeting molecules suffer is acting to rapidly degrade this fingerprint into a statistical distribution (6). Indeed, energy released into translation and rotation is nearly always (7, 8) relaxed into the solvent on the time scale of the reactive event itself, but vibrations can relax much more slowly. High-frequency vibrations like those involving hydrogen atom stretches in diatomic and triatomic molecules can take hundreds of picoseconds to relax (9, 10), so it should be possible to observe a vibrational distribution for a solution-phase reaction (6). However, there are few examples for a bimolecular reaction where this state distribution has even been measured (11).

The Polanyi rules provide insight into the origin of nonstatistical energy distributions in

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Crossing crowded dance floors. In the dance that makes up a chemical reaction, an existing bond is broken (here, the C-H of the ring-shaped cyclohexane molecule) and a new one formed (a new C-H in the HCN product). The hydrogen atom (white sphere) is transferred to the CN radical, $\text{CN}^\bullet + \text{C}_6\text{H}_{11} \rightarrow \text{HCN} + \text{C}_6\text{H}_{11}^\bullet$. (A) In the gas phase, the reactants collide unimpeded (the dance floor is clear). The straight red arrow shows a classical reactive trajectory superimposed

on the potential energy surface. The contour map is a function of the lengths of the C-H bond being formed (vertical axis) and the one being broken (horizontal axis). Because the transition state (X) is early, the downhill descent directs kinetic energy into the newly forming H-CN bond. (B) In solution, the reactants move more chaotically across a crowded dance floor. Greaves *et al.* show that despite solvent friction, much of the energy released is still directed into the H-CN bond.

terms of the location of the transition state (X) on the potential energy surface (see the figure, panel A), a map showing how the internal energy of the molecules changes with the position of the atoms. For a reaction where this barrier occurs “early”—the transition state looks more like the reactants—translational (rather than internal) energy is most effective at getting over the barrier. Downhill from the barrier, energy is released first into compressing the newly formed bond before the old bond is broken so the products are born with high stretching excitation. If the reaction releases a lot of energy and the barrier is early enough, an inverted population (like that created in the HF laser) will occur.

When solvent is added to this gas-phase picture, the frequent collisions between solvent and reactant in the entrance channel lead to more chaotic trajectories that might deprive the nascent products of state-specific energy disposal (see the figure, panel B). The close packing of the solvent should also provide mechanical and electrostatic friction (inset in the figure, panel B) that prohibits the reaction from delivering so much of its initial energy release into one specific mode. Greaves *et al.* build on pioneering experiments by Hochstrasser and co-workers where a free radical, $\text{CN}^\bullet$, strips a hydrogen atom from a solvent molecule (11). They replaced the reaction partner with cyclohexane, and by capturing broad sections of the infrared spec-

trum as a function of time after preparing the radical, they showed that the energy content in the C-H stretching vibration of the newly formed bond is not just slightly nonstatistical, as in the earlier work (6, 11), but has a strong population inversion (3). The time-resolved spectra also reveal excitation channeled into bending excitation of the product.

Although the solvent damped to some extent the channeling of energy into product vibration, for this particular reaction, the basic picture is little changed from that of the gas phase. The authors’ selection of a reaction with an isolated high-frequency stretching mode in the product, a very low barrier, and a flat energy landscape near the transition state were likely crucial in revealing Polanyi-type reaction behavior. This choice separates the time scales between reaction and slow product relaxation and may lead to insensitivity to the direction of approach to the transition state. The downhill descent driving energy into the newly formed H-CN bond must be faster than any friction can be developed by the solvent. Finally, contrary to what was inferred for the $\text{CN} + \text{HCCl}_3$ reaction (6, 11), solvation has not pushed the barrier any later along the reaction coordinate. Calculations presented by Greaves *et al.* support rather small changes to the reaction potential energy surface on solvation.

It remains to be seen then whether state-specific energy disposal in the liquid phase is

more general. Another intriguing possibility arising from this work is that rotational excitation surviving from the laser-induced dissociation (8) used to generate (12) the CN radicals here might be transmitted through the transition state and into the HCN bend, as Greaves *et al.* speculate. Perhaps other manifestations of the Polanyi rules, i.e., state-specific effects in the entrance channel of a reaction with a late barrier, may also be accessible with suitable preparation (13) for solution-phase bimolecular reactions.

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RETROSPECTIVE

Leon Knopoff (1925–2011)

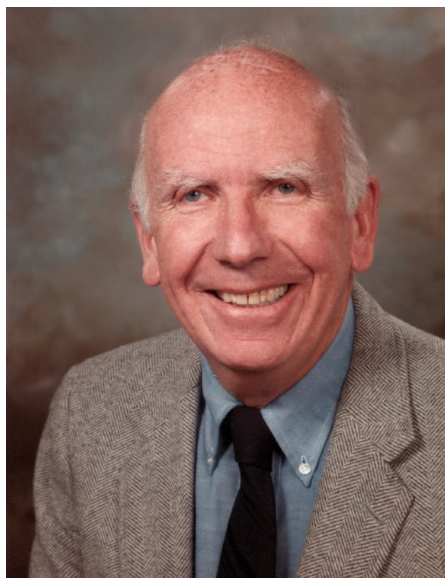
Paul M. Davis and David D. Jackson

Leon Knopoff, a preeminent seismologist, died at his home in Los Angeles, California, on 20 January. His extraordinary talents spanned fields as diverse as quantum mechanics and musicology, earning him a reputation as a Renaissance man of the modern era.

Knopoff was born in Los Angeles in 1925. He studied electrical engineering as an undergraduate and obtained his Ph.D. in physics and mathematics at the California Institute of Technology (Caltech) in 1949. The following year, he was recruited by Louis Slichter to the Institute of Geophysics at the University of California, Los Angeles (UCLA), as a research associate. He held faculty positions at Miami University in Ohio (1948–1950) and Caltech (1962–1963). Knopoff became a professor of Geophysics and Physics at UCLA, where he remained for 60 years. His interests in pattern recognition and music coalesced when, as an accomplished pianist and harpsichordist, he became a research musicologist at the UCLA Institute of Ethnomusicology shortly after it was formed in 1960.

Among many honors, Knopoff was elected to the U.S. National Academy of Sciences (1963) and the American Philosophical Society (1992), and as a fellow of the American Academy of Arts and Sciences (1965). He received the H. F. Reid medal of the Seismological Society of America, the gold medal of the Royal Astronomical Society, the Emil Wiechert medal of the German Geophysical Society, and a Docteur Honoris Causa from Université Louis Pasteur, Strasbourg. He particularly treasured four teaching awards from the UCLA Physics Department. Along with his many students and colleagues, we benefited from his extraordinary ability to teach complexity in the simplest of terms, with an infectious animation and the patience to impart understanding.

His encyclopedic memory and theoretical prowess earned him many first discoveries listed in more than 350 publications involving his 39 research students and 40 postdoctoral scholars. In 1956, Knopoff published a theoretical framework for the “double couple” model of an earthquake



(considers ground motion across a fault plane), which is now a standard approach used by engineers and seismologists to predict ground shaking. He showed how motion on a boundary, such as a seismic fault, is linked to displacements in a medium, such as Earth's crust, and demonstrated that the displacements are proportional to the velocity of slip across a fault plane.

In the 1960s, Knopoff's independent and collaborative work included developing computational approaches for understanding the dynamics of fault planes and the propagation of seismic waves. These laid down the models for using seismograms (recordings of ground motion as a function of time) to obtain both the earthquake mechanism and the velocity along the ray path. He not only pioneered the installation of temporary long-period seismograph stations in locations throughout the European Alps; he and colleagues Stephan Mueller and Walter Pilant were the first to perform digital processing of long-period seismograms in 1966. At the South Pole, Knopoff helped to develop and install an ultralong-period seismometer and was the first to measure solid Earth polar tides and vibrational modes, unaffected by Earth's rotation and elliptical shape. He used global seismographic data to define the main structures of plate tectonics, and showed that the oceanic lithosphere thins at mid-ocean ridges and lies above a decoupling zone between plates and the mantle below. In 1972, Knopoff was the first to show that

Interests in earthquake dynamics and music were driven by a seismologist's passion for understanding the physics behind pattern recognition.

unlike thin oceanic lithosphere, regions of stable continental crust (shields) possess very deep roots, or keels. These keels slow the motion of continental plates as they plough through the mantle. Modern plate models and geodetic measurements of plate motions support these pioneering models.

The range of Knopoff's theoretical advances covers nearly everything seismological, including diffraction, attenuation, creep, equations of state, scattering, cracked media, and dynamic crack propagation. He was one of the first to recognize the applicability to the earthquake problem of modern developments in nonlinear science such as chaos, strange attractors, fractality, and self-organized criticality. The highly cited model of interacting springs and blocks by Knopoff and Robert Burridge (1967) became the basis for simulating self-organization and chaos in the earthquake dynamical system. It predated by two decades such developments in physics.

With Yan Kagan, Knopoff developed the stochastic branching model of faulting that displays the clustering properties of earthquake catalogs, including foreshocks, aftershocks, and weak clustering of mainshocks. Again, he was years ahead of the now famous epidemic type aftershock sequences (ETAS) model. His mechanical theories and statistical analyses of earthquake catalogs have provided seismologists a framework for long-term forecasting of seismic hazard.

Another first was the discovery, with George Kennedy, of thermoluminescence dating of ancient pottery—a procedure widely used by archaeologists and art historians. Rocks and pottery contain small amounts of radioactive elements that decay over time. The electrons emitted during this process become trapped in the solid material, but when heated to about 350°C, are freed, emitting light. Age can thus be estimated according to the amount of light given off.

Leon Knopoff was a warmhearted man, very much at home climbing glaciers, hiking in the Sierras, advancing theoretical physics, and applying pattern recognition to melodic stylistic analysis or language. A remarkable polymath, he effectively spanned the “two cultures” of science and art, leaving an extraordinarily rich record of achievement and a cadre of students who continue his work.

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Experimental Philosophy and the Problem of Free Will

Shaun Nichols

Many philosophical problems are rooted in everyday thought, and experimental philosophy uses social scientific techniques to study the psychological underpinnings of such problems. In the case of free will, research suggests that people in a diverse range of cultures reject determinism, but people give conflicting responses on whether determinism would undermine moral responsibility. When presented with abstract questions, people tend to maintain that determinism would undermine responsibility, but when presented with concrete cases of wrongdoing, people tend to say that determinism is consistent with moral responsibility. It remains unclear why people reject determinism and what drives people's conflicted attitudes about responsibility. Experimental philosophy aims to address these issues and thereby illuminate the philosophical problem of free will.

Many central philosophical problems—such as problems concerning free will, morality, and consciousness—have their roots in our ordinary ways of understanding the world. It takes no special training to come to appreciate questions like “How can a material object be conscious?” or “Is morality only relative to culture?” Such problems resonate with common sense. These common-sense philosophical problems are also notorious for their resilience. Many of them stretch back to the earliest days of philosophy. The problems have persisted, and to a great extent, so have the techniques used to advance the inquiry. The central technique is careful and sustained thought, sharpened by dialogue with fellow philosophers.

Experimental philosophy is a recent movement that brings new techniques to bear on philosophical problems (1). The techniques are actually familiar experimental methods from social science, but the targets of these experiments are distinctively philosophical, like the common-sense philosophical problems surrounding free will, morality, and consciousness. Because these problems are grounded in common sense, experimental philosophers aim to diagnose the psychological origins of philosophical problems, and such diagnoses might indicate new avenues of treatment.

In some cases, a common-sense philosophical problem arises because it is difficult for us to make sense of the way the world works. For instance, it is difficult to understand, intuitively, how a physical thing like the brain can produce the conscious experience of tasting cantaloupe, which seems utterly different from anything physical (2). In other cases, common-sense philosophical problems arise because we seem to have conflicting intuitions. Here, free will provides a vivid example. On the one hand, it seems that, at the moment of making a decision about whether to stop writing (for instance), it is genuinely possible for one to freely decide either way, and

“... experimental philosophers aim to diagnose the psychological origins of philosophical problems.”

after the decision is made, it still seems that one could have decided something else, even if everything leading up to the decision had been exactly the same (3, 4). On the other hand, it also seems intuitive that when an event occurs, there should be a complete causal explanation for why it happened. Every event seems to be caused by the preceding events. It would be strange to say that the choice to continue writing just happened, with no causal explanation. The idea that everything that happens must have a causal explanation is central to determinism, the view that (roughly) every event is completely caused by what happened leading up to the event. Determinism is intuitively attractive, but it seems to conflict with the idea that, at the moment of a decision, it is genuinely possible to choose one way or the other (5, 6). It seems like something has to give, either our commitment to free will or our commitment to the idea that every event is completely caused by the preceding events.

Agency and Determinism

Philosophers have explored the apparent intuitive conflict between free will and determinism by invoking sophisticated logical arguments and semantic analyses (7, 8). Experimental philosophers focus instead on the psychological aspects of the problem. Are people really pulled in these

different directions? Do ordinary people really have intuitions at odds with determinism, as philosophers suggest? And if so, what is the ultimate psychological source of these intuitions?

A person typically will not regard her current action as free unless he or she feels like it is her own voluntary action. Researchers have proposed two very different factors that contribute to this sense of agency. According to one hypothesis, the internal motoric signals that cause behavior also generate a prediction about imminent bodily movement, and this prediction is compared to the actual sensory information of bodily motion. If the predicted movement conforms to the sensory information, then one gets the feeling of agency; otherwise the movement is likely to feel involuntary (9). Another proposal maintains that a person's sense of agency is affected by the presence of external

cues, such as the time interval between an external cue and a subsequent behavior (10). These internal and external cues both seem to contribute to the sense of agency (11).

The sense of agency is critical to any sense that one's current action is free, because an action will not feel free unless it feels like one's own action. But there is a further question about whether one's actions are regarded as undetermined. Developmental psychologists have recently shown that, in some circumstances, young children reason in a way that suggests a belief in determinism.

When observing physical events like a light going on, children expect there to be a causal explanation for the event. After they have observed a light going on when a switch is flipped, if the switch subsequently fails to turn on the light, young children reliably search for a causal explanation for why the light did not turn on (12). However, experimental philosophers have also found evidence suggesting that 3- to 5-year-old children reject determinism in the context of human action. With a child observing, an experimenter performed a simple action such as putting her hand in a box, and the child was then asked whether the experimenter could have done something else. The vast majority of children said that the person could have done something else (13, 14). Most children did not, however, say the same thing after observing a physical event like a ball rolling into the box. Rather, in that case, children denied that the ball could have done something else. The conflicting intuitions that give rise to the problem of free will may already be present at an early age.

A different way to assess whether people reject determinism is to present them with a non-technical description of a deterministic universe and then gauge their reaction. This method has now been employed in several studies on adults. In one study, a deterministic universe was char-

acterized as follows: “Everything that happens is completely caused by whatever happened before it. This is true from the very beginning of the universe, so what happened in the beginning of the universe caused what happened next, and so on right up until the present. For example, one day John decided to have French fries at lunch. Like everything else, this decision was completely caused by what happened before it. So, if everything in this universe was exactly the same up until John made his decision, then it had to happen that John would decide to have French fries” (15). After reading such a description, adult participants in the U.S. tended to reject the idea that our universe is like this, at least when it comes to human decision-making. Subsequent cross-cultural research finds the same pattern in Chinese, Indian, and Colombian populations (16).

These results from experimental philosophy confirm what many philosophers already maintained: that common sense is committed to indeterminism about decision-making (3–6). But the results underscore a puzzling aspect of this common-sense commitment. What leads people to reject statements of determinism? What are the psychological sources of this reaction? After all, determinism is a sophisticated theory of the universe. And it is likely that many of the participants in these experiments had never previously given determinism much thought. Thus, one goal of recent work has been to figure out why people tend to converge on the rejection of determinism.

One explanation with an impressive philosophical pedigree is that people reject determinism because introspection does not reveal a deterministic set of causes of action (3, 17, 18). Often when we introspect on our reasons for performing an action, the reasons we perceive do not univocally point to a particular action. Cognitive scientists widely agree that introspection fails to reveal all of the causal influences on our actions (19, 20). But it remains unclear whether this introspective limitation provides a complete explanation for why people reject determinism.

An alternative proposal focuses on the way people think about the self as an agent (21, 22). As noted above, research on the sense of agency shows that both internal and external cues contribute to the sense that “I” produced an action (9–11). But how do people think of the self that produces the action? Determinism might be counterintuitive because of the way people think of the self (23). A computer’s behavior is obviously a function of the computer program and the inputs. But the self, accord-

ing to this proposal, is conceived as something distinct from psychological mechanisms and inputs, something that can stand apart from those details and make an executive decision. If people think of the self as something apart from the inputs and mechanisms, then it is not surprising that they reject the idea that decisions are produced by deterministic mechanisms and processes.

Moral Responsibility and Determinism

Suppose it turns out that determinism is true. What does that mean in terms of whether people are morally responsible for their actions? Philosophers have long been divided on this question. One view, compatibilism, maintains that determinism is quite irrelevant to the issue of moral responsibility; i.e., our ordinary ways of thinking about responsibility are immune to any threat from determinism (24). The opposing view, incompatibilism, maintains that if determinism is true, no one is truly morally responsible (4, 5, 25).

Experimental philosophers have shown that ordinary people, like philosophers, offer conflicting views. In one experiment, participants were

presented with a description of a deterministic universe in which “Every decision is completely caused by what happened before the decision—given the past, each decision has to happen the way that it does.” After being presented with this description of determinism, one group of participants was asked whether it is possible for anyone to be morally responsible for their actions in such a universe. These participants tended to say that it is not possible to be morally responsible in that universe. That question about moral responsibility is, of course, pitched at an abstract level. Another group of participants was presented instead with a concrete case of a man who killed his family. That provoked a much different response. When presented with a concrete case of a man performing a reprehensible action, people tended to say that the man was fully morally responsible for his actions, even when set in a deterministic universe (Fig. 1A) (15). Indeed, concrete cases of bad behavior lead people to attribute responsibility, even when the action is caused by a neurological disorder (26). In contrast, when asked abstract questions about whether people in a deterministic universe should be blamed

for bad actions, people tend to say that they should not be blamed. Moreover, a study on a diverse set of cultures—including the U.S., China, and India—found no cultural differences on these kinds of questions. People in all cultures tended to maintain that in a deterministic universe, people would not be morally responsible for their actions (16).

Related results emerge when participants are presented with the vivid possibility that our own universe is deterministic. In one condition, participants are told that many eminent scientists are convinced that determinism is true in our world: “that every decision a person makes is completely caused by what happened before the decision—given the past, each decision has to happen the way that it does.” In the other condition, participants are told to imagine another universe in which many eminent scientists are convinced that determinism is true. In both conditions, they are then asked to consider whether, if those scientists are right, people are morally responsible. When imagining alternate universes, participants tend to give incompatibilist responses, saying that people are not morally responsible in that case. However, when led to consider our own universe as deterministic, participants were more likely to say that people would still be morally responsible (27, 28). Imagining a less distant scenario makes people give more compatibilist responses.

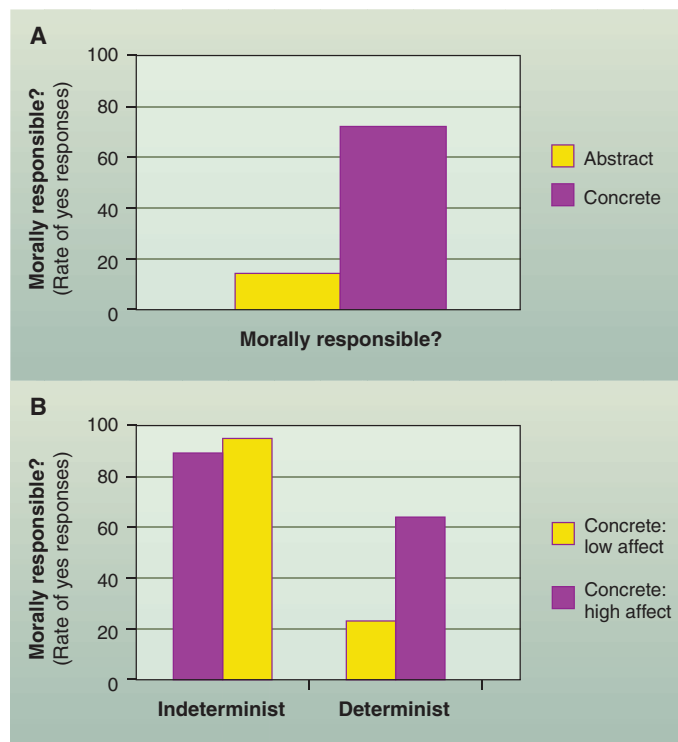


Fig. 1. (A) After reading a nontechnical description of determinism, one group of participants was asked an abstract question about whether it is possible to be morally responsible in such a universe. The other group was given a concrete scenario in which a particular person did something terrible in that universe, and they were asked whether that person is morally responsible. **(B)** After reading the description of determinism, participants were presented with either a concrete case of an emotionally upsetting (high-affect) transgression or a concrete case of an emotionally bland (low-affect) transgression. In half of the cases, the transgression was set in an indeterminist universe. In that case, participants were equally likely to attribute responsibility in both high- and low-affect conditions. In the other half of the cases, the transgression occurred in a determinist universe. In that case, participants were significantly more likely to attribute responsibility in the high-affect condition (12).

What explains these conflicting responses? One class of explanation holds that the responses in the abstract condition reveal the true common-sense view. When they are calm and collected, people acknowledge that determinism precludes responsibility. In contrast, when faced with cases that are concrete and close to home, our responses are biased by emotional and motivational factors. Our desire to blame the offender (29) drives us to blame, even under the assumption of determinism (15). Previous research indicates that emotional activation does affect attribution of blame; i.e., participants who have been made to feel negative emotion issue harsher judgments of blame (30). Additionally, there is reason to suspect that some of the manipulations in the free-will experiments do differentially trigger affect. For instance, concrete descriptions of reprehensible actions are likely to be more upsetting than abstract questions about moral responsibility. This might explain why people in the concrete condition are more likely to attribute responsibility; their emotions are driving their judgments of responsibility. Some evidence in favor of this interpretation comes from the fact that when participants in the determinist experiments are presented with an emotionally charged “high-affect” concrete transgression (i.e., rape), they are much more likely to attribute responsibility than when they are presented with a low-affect concrete transgression (i.e., tax evasion) (Fig. 1B) (15).

A very different explanation of people’s conflicting judgments focuses on the incompatibilist responses and maintains that these responses result from a misunderstanding about the nature of determinism (31, 32). The hypothesis maintains that if people think the world is deterministic, their conscious deliberations and other psychological processes are causally irrelevant. However, this is not what determinism entails. Determinism is consistent with the idea that behavior is produced (i.e., determined) by conscious psychological processes. To explore whether this kind of confusion affects responses, participants were presented with different versions of determinism. In one condition, behavior was said to be determined by neurological and chemical processes. In that case, participants drew incompatibilist conclusions, saying that people were not responsible if behavior is all caused by neurological and chemical processes. In the other condition, behavior was characterized as being determined by psychological processes. In that case, participants tended to give compatibilist responses, maintaining that people are responsible even if their actions are determined by their psychological states (31). This suggests that determinism itself might be less threatening to our ordinary ideas of moral responsibility, so long as the determining causes are our psychological states and processes.

Potential Implications

Just as philosophers have advocated diametrically opposed views on whether determinism

undermines responsibility, ordinary people offer conflicting views on the matter. In some cases, people tend to say that determinism would undermine responsibility; in other cases, they tend to regard determinism as compatible with responsibility. Experimental philosophy holds out the promise of explaining why we are pulled in different directions. The extant work suggests that people are pulled in different directions because different mental mechanisms are implicated in different conditions. As noted above, one hypothesis is that incompatibilist responses—that responsibility is not compatible with determinism—are favored by mental mechanisms devoted to unemotional abstract reasoning. In contrast, compatibilist views seem to be favored when emotional attitudes are triggered by concrete scenarios. If something along these lines is right, then this would help explain the persistence of the philosophical dispute over free will and moral responsibility. Part of the reason that the problem of free will is so resilient is that each philosophical position has a set of psychological mechanisms rooting for it (33).

In addition to diagnosing the resilience of the problems, experimental philosophy might also contribute to solving the problems, for we might discover that some of the natural responses are the product of error or bias. If so, we would have reason to disregard the responses rooted in error. On this topic, the current work is quite divided. One view is that people’s incompatibilist responses are the result of a deep misunderstanding of the nature of determinism (32). On this view, people mistakenly conclude that determinism entails that our psychological attitudes and values have no effect on decisions. As a result, people’s fear of determinism is ill founded. A very different account holds that people’s emotional reactions to reprehensible actions swamps their rational ability to recognize the threat that determinism poses for responsibility. When people are allowed to assess the problem of free will and responsibility in a calm fashion, they tend to judge that responsibility is indeed threatened by determinism (15). If this is the correct psychological explanation of the response, then people are right to think that determinism threatens moral responsibility.

Experimental philosophy is also poised to shed light on the belief in free will itself. If we identify why people think that their choices are not determined, we will be in a better position to evaluate that belief. A psychological account of the genesis of free-will beliefs probably cannot directly show that such beliefs are true or false. But knowing why people believe in free will might well put us in a position to evaluate whether or not people’s belief in free will is justified (34).

This Review has focused on the experimental philosophy of free will, but it is really a case study that gestures to the broader promise of experimental philosophy. As noted at the beginning of this paper, several other central philosophical

problems have their roots in everyday thought. People seem to be torn on whether morality has an objective footing or is merely relative to culture. What factors pull in those different directions? People also find it difficult to make sense of the idea that a physical object such as the brain could produce conscious experiences. Why is that connection challenging to understand? If we can comprehend the psychological sources of these kinds of problems, we might be in a better position to resolve or defuse the problems.

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Atmospheric P_{CO_2} Perturbations Associated with the Central Atlantic Magmatic Province

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The effects of a large igneous province on the concentration of atmospheric carbon dioxide (P_{CO_2}) are mostly unknown. In this study, we estimate P_{CO_2} from stable isotopic values of pedogenic carbonates interbedded with volcanics of the Central Atlantic Magmatic Province (CAMP) in the Newark Basin, eastern North America. We find pre-CAMP P_{CO_2} values of ~2000 parts per million (ppm), increasing to ~4400 ppm immediately after the first volcanic unit, followed by a steady decrease toward pre-eruptive levels over the subsequent 300 thousand years, a pattern that is repeated after the second and third flow units. We interpret each P_{CO_2} increase as a direct response to magmatic activity (primary outgassing or contact metamorphism). The systematic decreases in P_{CO_2} after each magmatic episode probably reflect consumption of atmospheric CO_2 by weathering of silicates, stimulated by fresh CAMP volcanics.

Large igneous provinces (LIPs) are geologically rapid episodes of extensive volcanism, often flooding vast oceanic or continental regions with several million cubic kilometers of lava (1). In particular, continental flood basalts have the potential to directly perturb Earth's climate system through the emission of gasses to the atmosphere: most notably, SO_2 and CO_2 , which together may result in an immediate (1- to 10-year) cooling (2, 3), followed by a longer-term (10^2 - to 10^5 -year) warming (4). Of these, only CO_2 has the potential to influence climate on both short and long time scales because of its relatively long atmospheric residence time and effectiveness as a greenhouse gas, leading some to conclude that CO_2 is the primary driver of Phanerozoic climate (5).

If the concentration of atmospheric CO_2 exerts an influence on climate over such broad time scales, what are the effects of a LIP on this essential parameter of the carbon cycle? Although the potential radiative effects of LIP CO_2 de-

gassing on the million-year scale have been considered inconsequential (6, 7), shorter (10^4 - to 10^5 -year)-time scale reconstructions of atmospheric partial pressure of CO_2 (P_{CO_2}) before and after LIP eruptions have not been systematically determined because of inadequate chronostratigraphic resolution in most settings (8, 9). Consequently, the direct P_{CO_2} effect of a LIP remains untested empirically.

Intriguingly, LIP volcanism is often temporally associated with mass extinction events throughout Earth's history (10). The three largest continental LIPs of the Phanerozoic are the Siberian Traps, the Central Atlantic Magmatic Province (CAMP), and the Deccan Traps, each of which is linked to one of the "Big 5" Phanerozoic mass extinctions [the end-Permian, end-Triassic, and the Cretaceous–Paleogene events, respectively] (11, 12). Though attempts have been made to estimate the gaseous emissions attributable to the Deccan (6, 13, 14) and Siberian (15, 16) traps, it is difficult to demonstrate causality because the uncertainties in correlating these P_{CO_2} estimates from afar to the volcanic stratigraphy itself are usually no better than the turnover time of an atmospheric P_{CO_2} perturbation (17). Of these, only the CAMP is sequenced in high-resolution, temporally continuous sediments that

contain paleosols appropriate for estimating P_{CO_2} and have a well-established chronology (18, 19) and extinction level.

Extrusives from the CAMP (20) are preserved in direct stratigraphic succession with cyclic continental sediments in the Newark Basin of eastern North America (Fig. 1). Milankovitch cyclostratigraphy of the primarily lacustrine sediments interbedded within the CAMP extrusives have yielded precise age control (to the level of orbital precession) and an estimated total volcanic duration of $\sim 600 \pm 20$ thousand years (ky) (21, 22). In this same Newark Basin section, palynofloral evidence of the end-Triassic extinction (ETE) is found stratigraphically just below the first of the CAMP volcanics, preceding the magmatism by ~ 20 ky [(23), see (24) for review]. Also interspersed throughout these sediments, and often forming from CAMP lava flows themselves, are pedogenic carbonate-bearing paleosols (Fig. 2, A and B), which can be used to estimate ancient atmospheric P_{CO_2} (25). Thus, the Newark stratigraphy is ideally situated to directly test the P_{CO_2} effect of a LIP. Previous attempts at reconstructing the P_{CO_2} effect associated with CAMP extrusives had very sparse sampling resolution (26) or had to rely on imprecise long-distance correlation (8, 9).

We use $\delta^{13}C$ measurements of pedogenic carbonate nodules from paleosols stratigraphically distributed before and after each extrusive unit to generate a high-resolution P_{CO_2} record through the Newark Basin CAMP sequence (Fig. 1). The extrusion of ~ 2 to 4×10^6 km³ of volcanics (27, 28) in less than 1 million years (My) implies a measurable effect on atmospheric P_{CO_2} , which our temporal resolution should allow us to detect. According to the model of Dessert *et al.* (17) scaled to the Deccan Traps, the transient increase in P_{CO_2} is on the time scale of the eruptions, after which continental silicate weathering should lower P_{CO_2} to pre-eruption levels in ~ 1 My.

Estimating P_{CO_2} from pedogenic carbonates. Organic and inorganic carbon isotope measurements on paleosols from outcrop—and from multiple, stratigraphically overlapping cores taken by the Army Corps of Engineers (ACE) through the extrusive interval (fig. S1) (29)—are used as inputs into the diffusion model of Cerling (30)

$$C_a = S(z) \frac{\delta_s - 1.0044\delta_p - 4.4}{\delta_a - \delta_s} \quad (1)$$

Fig. 1. (A) Stratigraphy and lithology (22, 34, 41) of the upper Newark Basin stratigraphic section, based on assembly of a series of short cores taken by the ACE covering the extrusive interval in high resolution (49), with substantial overlap both internally and with the Newark Basin Coring Project (NBCP) Martinsville core (19, 22) and outcrop. Note that the ETE event (red) is several meters below the equivalent of the Orange Mountain Basalt (OMB, the first flow unit) in the Jacksonwald section of the Newark Basin (24). Stratigraphic thickness is scaled arbitrarily from the base of the laterally extensive OMB. J, Jurassic; Tr, Triassic; UU through MM are stratigraphic members. (B) Profile-equilibrated mean $\delta^{13}C$ values of pedogenic carbonate in the Newark stratigraphic section. Error is $\pm$ SD of mean (Fig. 2 and table S1) (29). Circles, samples from core;

squares, outcrop. PDB, Pee Dee belemnite. (C) Measured $\delta^{13}C$ values of preserved soil organic matter from clay linings or as close to the paleosol surface as possible. (D) Results of the pedogenic carbonate paleobarometer based on the input variables from (B) and (C) at 25°C. The concentration of respired CO_2 in the soil [$S(z)$] was estimated to be 3000 ± 1000 ppm (error bars), corresponding to a plausible range for midproductivity tropical soils and probably encompassing the range of calculated atmospheric P_{CO_2} values. Carbon-cycle perturbations are built into the model because the carbon isotopic ratio of the atmosphere (δ_a) is calculated from the measured $\delta^{13}C_{org}$ by: $\delta_a = (\delta^{13}C_{org} + 18.67)/1.10$ (32), which assumes consistent fractionation by photosynthesis [see (29) and table S1 for numerical values].

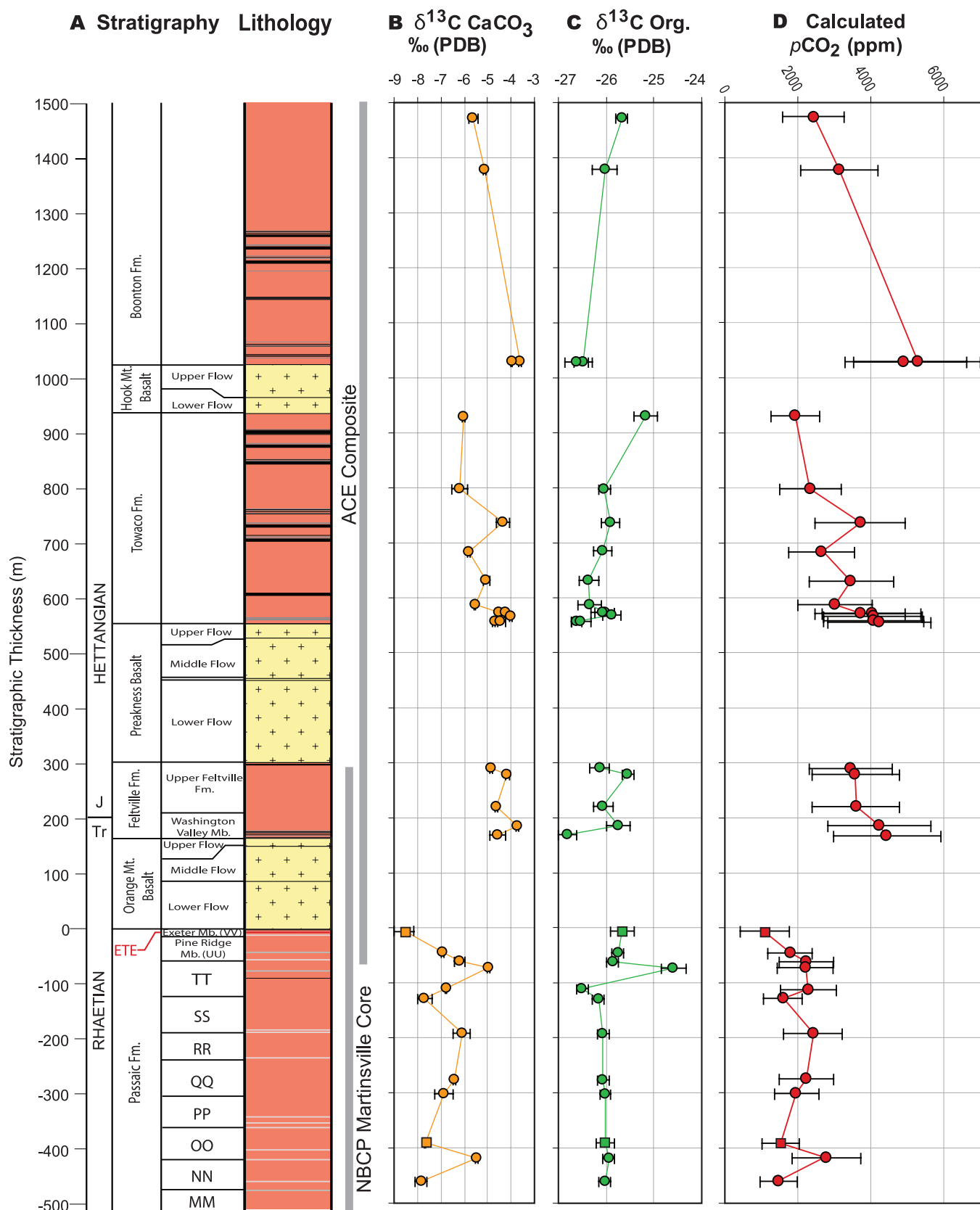
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where C_a is the concentration of atmospheric CO_2 , $S(z)$ is the concentration of CO_2 due to respiration of soil organic matter, δ_s is the $\delta^{13}\text{C}$ of soil CO_2 , δ_ϕ is the $\delta^{13}\text{C}$ of soil-respired CO_2 , and

δ_a is the $\delta^{13}\text{C}$ of atmospheric CO_2 . All δ values are relative to Vienna Pee Dee belemnite (VPDB). The temperature of calcite precipitation is set at 25°C , relating the carbon isotopic ratio of soil

carbonate (δ_{cc}) to δ_s (29). As an independent objective metric of soil applicability, multiple (three or more) down-profile δ_{cc} measurements were made on each paleosol to reproduce the



expected exponential decrease toward stabilization with depth (Fig. 2) (29, 31). Using the mean of these soil-equilibrated measurements ensures that the mixing between the atmospheric and soil-respired reservoirs is at equilibrium with respect to the diffusion model. The measured carbon isotopic ratio of soil organic matter ($\delta^{13}\text{C}_{\text{org}}$) is related directly to δ_{p} (29). Carbon-cycle perturbations are built into the model because the carbon isotopic ratio of the atmosphere (δ_{a}) is calculated from the measured $\delta^{13}\text{C}_{\text{org}}$ by assuming consistent fractionation by photosynthesis: $\delta_{\text{a}} = (\delta^{13}\text{C}_{\text{org}} + 18.67)/1.10$ (32). We use an $S(z)$ value of 3000 ± 1000 parts per million (ppm), appropriate for soils developed in a semi-arid climate with moderate productivity (30, 33).

Atmospheric P_{CO_2} estimates in superposition with CAMP basalts. Pedogenic carbonates from the upper Passaic Formation deposited before the first CAMP volcanic unit (Orange Mountain Basalt) have $\delta^{13}\text{C}$ values generally below -5 per mil (‰) VPDB, with ± 1.5 ‰ variability (Fig. 1B). The carbon isotopic composition of soil organic matter ($\delta^{13}\text{C}_{\text{org}}$) is remarkably stable around -26 ‰ (Fig. 1C), consistent with but less variable overall than results from independent analyses of wood and total organic carbon from elsewhere in the Newark and Hartford stratigraphy (34). Model results of the pedogenic carbonate paleobarometer yield P_{CO_2} estimates with a mean of ~ 2000 ppm [$S(z) = 3000$ ppm] in the pre-CAMP stratigraphy (Fig. 1D). These estimates from over 500 m of the uppermost Passaic Formation, deposited over ~ 2.5 My of the latest Triassic (18, 19), show internal consistency between outcrop and between cores and, with the tight stratigraphic resolution, suggest that there are no major CO_2 -producing magmatic events before the observed Orange Mountain Basalt. This stable P_{CO_2} background is probably a measure of equilibrium continental silicate weathering in the Late Triassic.

The ~ 2000 -ppm pre-CAMP P_{CO_2} baseline found in this study is not inconsistent with widely (geographically and stratigraphically) dispersed pedogenic carbonate estimates from elsewhere in the Newark Supergroup (26, 35) or reconstructions from the Late Triassic Petrified Forest section (Fig. 3) (8). However, the Petrified Forest section lacks CAMP volcanics and can be correlated to the Newark succession only at the stage level (36), in which case the Rhaetian samples most likely overlap with the 2.5-My pre-CAMP samples of this study but show considerably greater variability (500 to 3500 ppm) (Fig. 3). The high variability in the Petrified Forest section is probably due to the lack of down-profile isotope measurements and the use of temperatures estimated from the $\delta^{18}\text{O}$ of pedogenic calcite, which lacks a reliable calibration for use in the paleo record (37).

The carbon isotope value of pedogenic carbonate formed directly on top of the Orange Mountain Basalt (first flow unit) is enriched in ^{13}C by 1 to 2‰ above the pre-CAMP background (Fig. 1B). This increase and similar in-

creases above the Preakness and Hook Mountain Basalts (the second and third flow units, respectively) reflect the increased influence of the atmospheric reservoir on soil carbonate formed at depth. Stratigraphically, above each flow unit, the carbon isotope composition of soil organic matter decreases by ~ 0.5 to 1.0 ‰, followed by a general return of $\delta^{13}\text{C}_{\text{org}}$ values to around -26 ‰, but with increased variability (Fig. 1C). The $\delta^{13}\text{C}$ values of pedogenic carbonate and soil organic matter directly above the Preakness Basalt have particularly good reproducibility between several laterally equivalent individual cored sections, allowing confidence in these measurements. Those soils formed on top of the Orange Mountain Basalt (Fig. 2B) show an increase in P_{CO_2} to 4400 ppm (Fig. 1D), which amounts to a doubling of P_{CO_2} above the pre-CAMP baseline. In every case, pedogenic carbonate samples of soils formed on the tops of the basaltic units yield P_{CO_2} estimates that are distinctly higher than the immediately pre-eruptive background levels (Fig. 1): 4200 ppm on top of the Preakness Basalt compared with 3000 ppm in the uppermost portion of the underlying Feltville Formation, and

5000 ppm directly on the Hook Mountain Basalt compared with 2500 ppm in the uppermost portion of the underlying Towaco Formation. This pattern suggests that the volcanism associated with each lava-flow unit had a direct effect on atmospheric P_{CO_2} by ~ 2000 ppm and was virtually immediate [to within the resolution of orbital precession (~ 20 ky)].

Chronostratigraphic control of P_{CO_2} estimates.

A previous low-resolution reconstruction from the Newark Supergroup (two localities over 10 My) seemed to suggest relatively stable P_{CO_2} levels across the CAMP interval (26) and, when compared to our study, underscores the difficulty in attempting to capture a transient perturbation of the carbon system without adequate chronostratigraphic control (Fig. 3) (38, 39). The stratigraphic level of the McCoy Brook sample of Tanner *et al.* (26) from the Fundy Basin is not known precisely (40), and it is quite possible that the soil formed long enough after emplacement of the North Mountain Basalt (correlative to the Orange Mountain Basalt) (41) that the full P_{CO_2} increase was not recorded. As demonstrated by this study, capturing such a transient signal

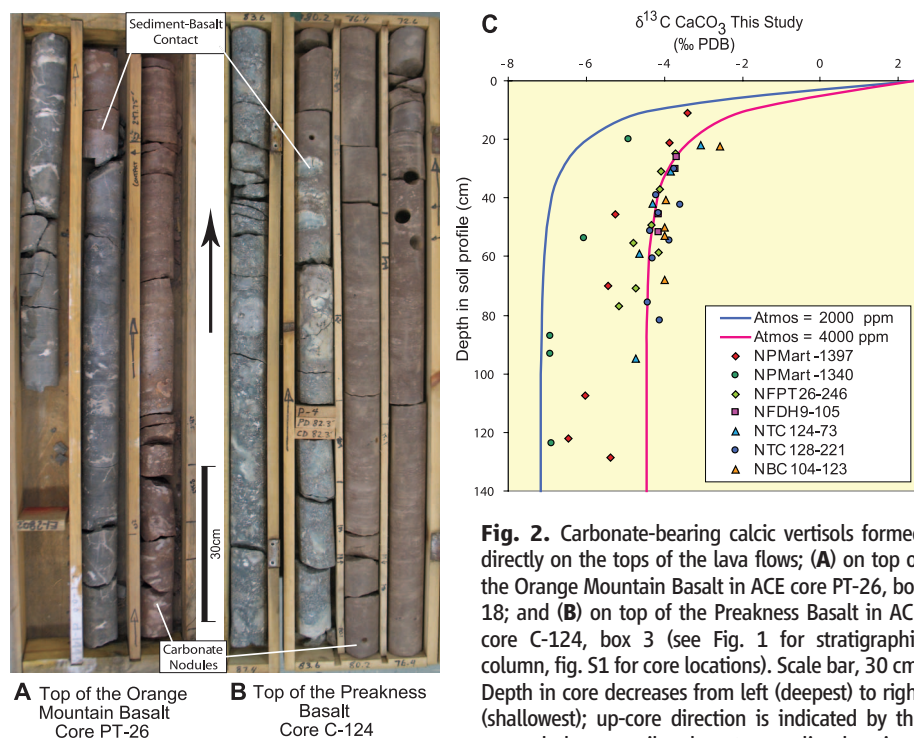


Fig. 2. Carbonate-bearing calcic vertisols formed directly on the tops of the lava flows; (A) on top of the Orange Mountain Basalt in ACE core PT-26, box 18; and (B) on top of the Preakness Basalt in ACE core C-124, box 3 (see Fig. 1 for stratigraphic column, fig. S1 for core locations). Scale bar, 30 cm. Depth in core decreases from left (deepest) to right (shallowest); up-core direction is indicated by the arrow; holes are soil-carbonate sampling locations.

Such superposition removes any stratigraphic uncertainty from these P_{CO_2} estimates with regard to the flow units themselves. (C) Down-soil $\delta^{13}\text{C}$ profiles of pedogenic carbonates (δ_{c}) from representative paleosols in the Passaic, Feltville, Towaco, and Boonton Formations used in this study (symbols encompass analytical error), compared with the δ_{c} predicted by the diffusion model at atmospheric CO_2 concentrations of 2000 and 4000 ppm (blue and pink lines, respectively) [after (31)]. For this exercise only, atmospheric $\delta^{13}\text{C}_{\text{CO}_2}$ was set to -6.5 ‰, and soil $\delta^{13}\text{C}_{\text{org}}$ was set to -26.5 ‰, with an exponential production function and characteristic depth of production at 15 cm [see (29) for description of other parameters]. For all P_{CO_2} estimates made in this study, the $\delta^{13}\text{C}_{\text{org}}$ was measured directly and used as a model input. Soil carbonate above 20 cm in the profile was rare. Note that stabilization of measured δ_{c} is often well below 50-cm soil depth. Using the mean of these depth-stabilized measurements ensures the mixing between the atmospheric and soil-respired reservoirs at equilibrium with respect to the diffusion model. Photographs of the soils used in (C), from samples NFPT26 and NTC124, are shown in (A) and (B).

requires <100-ky sampling resolution with respect to the volcanics. Other attempts to estimate P_{CO_2} over this interval, most notably the approximations using stomatal densities of leaf fossils from end-Triassic event boundary sections in Greenland and Sweden (9), indicate a doubling to tripling of P_{CO_2} from 800 to 2100 ppm (Fig. 3). These stomata-based estimates are substantially lower than those found here, but the stomata proxy is thought to underestimate P_{CO_2} (42), in which case a reported doubling to tripling broadly agrees with our findings and is corroborated by other cuticular estimates (43).

If the increase in P_{CO_2} after each major basaltic unit (Orange Mountain, Preakness, and Hook Mountain Basalts) can be ascribed to

episodes of magmatic activity, which are likely to be very short (22), then the relatively high-resolution record of P_{CO_2} taken as a whole seems to require just the three distinct episodes of volcanism. This does not necessarily imply that the local thicknesses of the CAMP volcanics in the Newark are representative of the global volume of basalt produced by each flow unit. For example, although the Newark and Hartford Basins show three distinct volcanic episodes in nearly identical chronostratigraphic sequence (41, 44), the thicknesses of the correlative flow units vary by a factor of 2. Nonetheless, the observed magnitude and singularity of the P_{CO_2} response to each flow unit in the Newark Basin implies that the magmatic events that produced them were

regionally extensive and voluminous, because atmospheric CO_2 is globally homogeneous on the circulation time of the atmosphere. Although the second episode of magmatism produced the thickest lava flow unit (Preakness Basalt) in the Newark Basin, there is only a relatively small corollary increase in P_{CO_2} . Interestingly, a middle flow unit equivalent to the Preakness Basalt is not present in the South Atlas region of Morocco, whereas the uppermost flow unit there (Recurrent Basalt) is stratigraphically and geochemically equivalent to the Hook Mountain Basalt in the Newark Basin (41). Although the Hook Mountain Basalt is locally thin in the Newark, the associated P_{CO_2} increase is one of the largest recorded, implying that greater unrecorded volumes were erupted elsewhere. This body of evidence demonstrates the global applicability of the P_{CO_2} findings reported here; together, they make a compelling case that what has been recorded in the Newark Basin is a reasonable representation of a global sequence of events.

To gauge the plausibility of a volcanic CO_2 source, we can compare the CO_2 efflux potential of the CAMP basalt volume to the effect observed in the Newark. Assuming a total CAMP volume of $2.4 \times 10^6 \text{ km}^3$ (27) and a volcanic efflux of $1.4 \times 10^{10} \text{ kg of } CO_2 \text{ per km}^3$ (7), we estimate a total CO_2 degassing potential of $3.36 \times 10^{16} \text{ kg } CO_2$. We focus on the lower volcanic unit (represented by the Orange Mountain Basalt in the Newark Basin), for which the pre-eruption background P_{CO_2} is well established. That initial pulse of activity represents roughly one-third of the total CAMP volume and, therefore, could have produced $1.12 \times 10^{16} \text{ kg of } CO_2$, amounting to a $\sim 1400\text{-ppm}$ increase in P_{CO_2} (at $7.82 \times 10^{12} \text{ kg } CO_2 \text{ per ppm}$). This instantaneous approximation is of the same order and within the error of our observed $\sim 2000\text{-ppm}$ P_{CO_2} increase after the first major episode of CAMP volcanism, implying that the volcanic release of CO_2 was extremely rapid, which is consistent with the sporadic presence of only very thin and discontinuous sedimentary strata between flows (21, 41). However, this does not preclude that a major component of the observed P_{CO_2} increase is contact metamorphic in origin (45), which is hinted at by the small decrease in $\delta^{13}C_{OM}$ above each flow unit.

The influence of basalt weathering. An intriguing phenomenon recorded by the Newark Basin paleosol sequence is the gradual apparent decrease in P_{CO_2} over time scales of 10^5 years after each successive episode of volcanism (Fig. 4). For example, elevated P_{CO_2} values just after the Preakness Basalt in the $\sim 300\text{-ky}$ -long Towaco Formation have nearly returned to pre-eruptive levels by the emplacement of the succeeding Hook Mountain Basalt. We attribute the systematic decrease in P_{CO_2} as the enhanced response of continental silicate weathering consuming each volcanic input of CO_2 (46). Given the vast aerial extent of CAMP extrusive activity (27), it is plausible to attribute the rapidity of the decrease in atmospheric CO_2 to consumption by hydrolysis

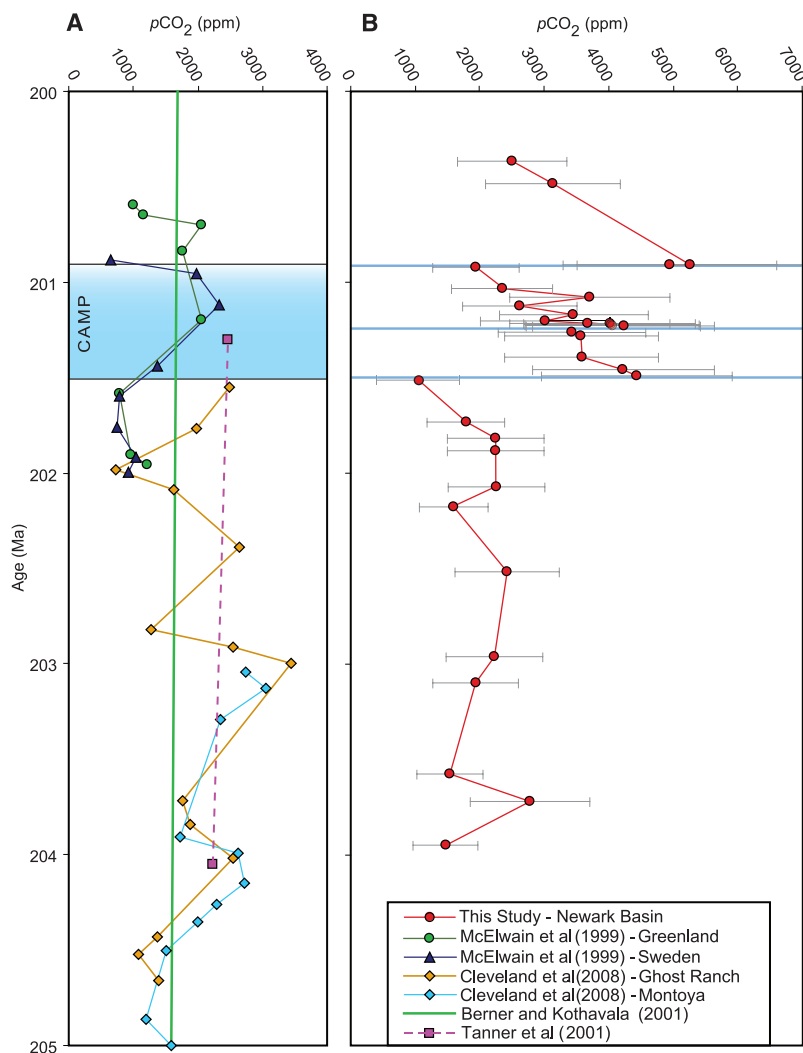


Fig. 3. (A) Atmospheric P_{CO_2} estimates previously made on Late Triassic and Early Jurassic sections using low-resolution pedogenic carbonates from other basins in the Newark Group (26) and the Rhaetian data of Cleveland *et al.* from the Petrified Forest section (8), stomatal densities from sections in Greenland and Sweden (9), and geochemical modeling (50). Approximate placement of the CAMP is shown in blue. The McElwain data were placed using the magnetic stratigraphy of Kent and Clemmensen (51) and Whiteside *et al.* (34). **(B)** P_{CO_2} estimates of this study with time [error bars are $S(z) = 3000 \pm 1000 \text{ ppm}$]. The samples taken in this study are from the same series of ACE cores used to construct a time sequence through the magmatic interval in the Newark Basin (22). The heavy blue lines indicate the temporal placement (and duration) of each CAMP flow unit. Ma, million years ago.

of the CAMP volcanics themselves, especially in the tropical humid belt where the engines of continental weathering are most effective (47). Geochemical modeling of the period after emplacement of the Deccan LIP and its corresponding CO_2 input show a similar exponential decrease in Pco_2 due to consumption by weathering (Fig. 4) (17). Marine osmium isotope evidence also indicates that an increase in continental weathering followed the CAMP interval (48), lending credibility to a weathering hypothesis. Though the CAMP data correspond well to the initial stages of this modeled decrease, the uppermost portion of the Newark Basin section

is truncated, so the full extent of this relation cannot be evaluated here.

Implications for the end-Triassic extinction.

Neither the Feltville nor Towaco Formations show evidence of Pco_2 changes that can be associated with magmatic events other than those directly related to the observed volcanics. Similarly, the stability of Pco_2 estimates in the Pre-CAMP Passaic Formation leaves the ETE without an obvious Pco_2 precursor. However, the youngest sample in the pre-extrusive section (Exeter Member, Fig. 1) formed in a soil predating the first flow unit by only ~20 ky. This soil occurs two meters below the clay layer con-

taining palynofloral evidence for the ETE in the same exposure, which itself predates the observed onset of volcanism in the Newark by ~19 ky (24). At these sedimentation rates, the uppermost paleosol sample that we studied in the Newark probably pre-dates the ETE by perhaps as little as ~1 ky. Therefore, it is possible that a pulse of CAMP volcanism and an attendant rapid rise in atmospheric Pco_2 with associated climatic implications occurred within the ~20-ky paleosol sampling gap before the age-equivalent of the Orange Mountain Basalt but remain undocumented. Nonetheless, the tight stratigraphic constraint implies that whatever phenomenon caused the ETE must have been very abrupt (occurring within a narrow thousand-year window) or have had minimal effect on atmospheric Pco_2 if it occurred earlier.

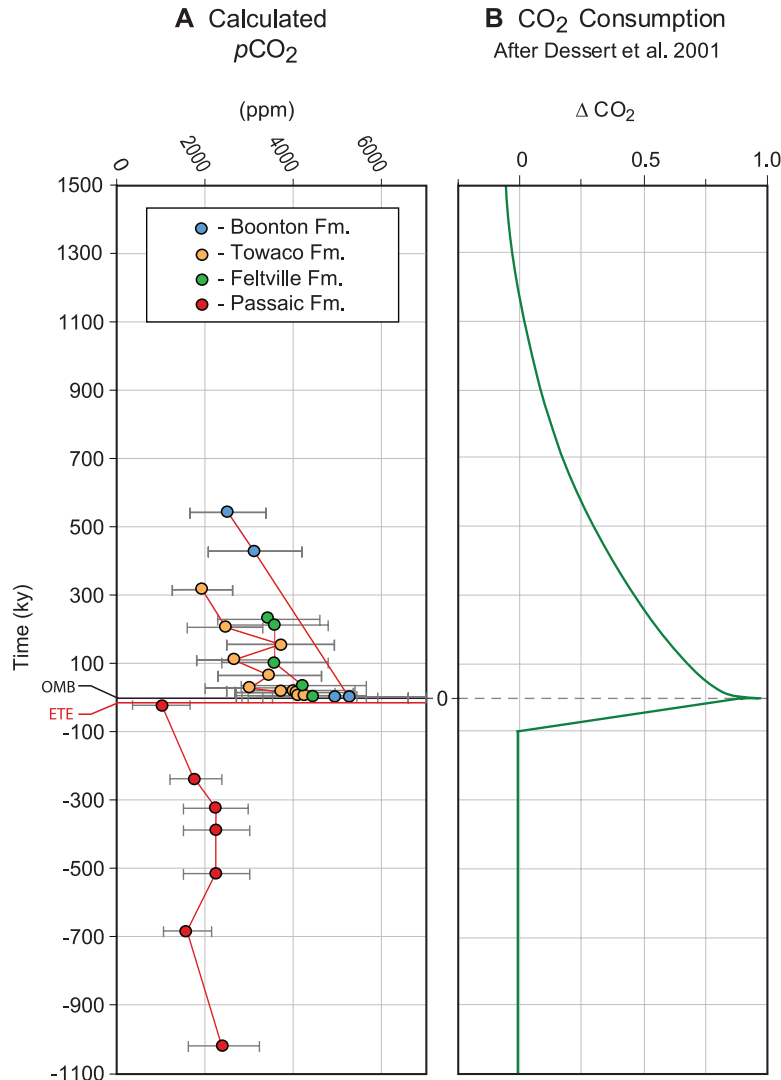


Fig. 4. (A) Calculated Pco_2 of this study versus time relative to the top of the Orange Mountain Basalt in the Newark Basin (the first CAMP flow unit). The Pco_2 estimates after each successive flow unit in the Newark have been normalized to the initial extrusive event for comparison to a silicate weathering model [colored symbols; error bars are $S(z) = 3000 \pm 1000$ ppm]. **(B)** Modeled CO_2 consumption due to weathering (17) after emplacement of the Deccan Traps. In the model, a pulse of CO_2 was added to the atmosphere (over 100 ky) accompanying the extrusion of the Deccan LIP. The change in Pco_2 represents the remaining fraction of the total Pco_2 increase in this particular modeling scenario. The abrupt increase of Pco_2 and subsequent postextrusive drawdown found in response to the CAMP volcanism in this study (A) is remarkably similar to the modeling results of Dessert *et al.* (17), bearing in mind the much longer interval of igneous activity assumed in the model.

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Supporting Online Material

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CRYPTOCHROME Is a Blue-Light Sensor That Regulates Neuronal Firing Rate

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Light-responsive neural activity in central brain neurons is generally conveyed through opsin-based signaling from external photoreceptors. Large lateral ventral arousal neurons (ILNvs) in *Drosophila melanogaster* increase action potential firing within seconds in response to light in the absence of all opsin-based photoreceptors. Light-evoked changes in membrane resting potential occur in about 100 milliseconds. The light response is selective for blue wavelengths corresponding to the spectral sensitivity of CRYPTOCHROME (CRY). *cry*-null lines are light-unresponsive, but restored CRY expression in the ILNv rescues responsiveness. Furthermore, expression of CRY in neurons that are normally unresponsive to light confers responsiveness. The CRY-mediated light response requires a flavin redox-based mechanism and depends on potassium channel conductance, but is independent of the classical circadian CRY-TIMELESS interaction.

The *Drosophila melanogaster* circadian clock circuit is composed of 140 to 150 neurons in the central brain and includes PIGMENT-DISPERSING FACTOR (PDF)-expressing lateral ventral neurons. The large lateral ventral neurons (ILNvs) are arousal neurons (1–3) and increase spontaneous action potential firing in response to light (4), whereas the small lateral ventral neurons (sLNvs) are critical for circadian function (5). Light resets the circadian clock via two mechanisms (6): rhodopsin-based external photoreceptors [the compound eye, ocelli, and the Hofbauer-Buchner (HB) eyelet] and the blue-light photopigment CRYPTOCHROME (CRY). *Drosophila* CRY is best known for its light-activated targeting of TIMELESS (TIM) for degradation, resetting the clock (7–9). External photoreceptors and CRY entrain the

Drosophila circadian circuit at vanishingly low light levels (10, 11). CRY also mediates magnetosensitivity in flies and butterflies (11–13).

In addition to the circadian molecular clock, membrane excitability is a key component of normal maintenance of circadian rhythms (14). Electrophysiological characterization of the s- and ILNvs has shown that their membrane properties are circadian-regulated outputs as well. Spontaneous firing frequencies are higher during the early day, gradually drop until dusk, and then rise again through the course of the night (1, 15). Additionally, the ILNv spontaneous firing frequency elevates 20 to 200% in response to moderately bright light (4). Given the plurality of light inputs to the ILNv, we investigated the ILNv electrophysiological light response (16) and found that the response is due to CRY acting by a cell-autonomous, redox-based mechanism, independent of CRY-TIM interactions, which requires the conductance of membrane potassium channels. Furthermore, ectopic expression of CRY optogenetically confers

electrophysiological light responsiveness to neurons that ordinarily do not respond to light.

Results. Both tonic and burst firing ILNvs recorded in the whole-cell current clamp configuration in an acutely dissected whole-brain preparation from flies expressing the *pdf**GAL4* driver and green fluorescent protein (GFP)-tagged nonconducting UAS-dORK, a *Drosophila* membrane-delimited potassium channel (4, 14), (*pdf**GAL4*-NC1-GFP) under dark conditions (>0.02 mW/cm²) immediately increased their firing rate and their resting membrane potential in response to moderate-intensity white light (4 mW/cm²) (Fig. 1A, top) or high-intensity blue light (19 mW/cm²) from a mercury light source (450 to 490 nm) (Fig. 1A, bottom), then rapidly returned to baseline firing rate upon return to darkness. The strength of the firing frequency ILNv light response, expressed here as the firing frequency with the lights on divided by the firing frequency with the lights off (FF on/FF off), varied with light intensity, exhibiting significantly higher firing frequency during lights on compared with lights off at intensities of 2 to 3 mW/cm² or higher (Fig. 1B). FF on/off for 19 mW/cm² was 1.62 ± 0.14 ($n = 11$) for 4 to 5 mW/cm² was 1.51 ± 0.15 ($n = 18$), for 2 to 3 mW/cm² was 1.39 ± 0.06 ($n = 68$), for 1 to 2 mW/cm² was 1.18 ± 0.02 ($n = 27$), for 0.6 mW/cm² was 1.23 ± 0.06 ($n = 16$), and for 0.3 mW/cm² was 1.10 ± 0.04 ($n = 13$). Light responses to intensities of 19 mW/cm², 4 to 5 mW/cm², and 2 to 3 mW/cm² were significantly different from 1 to 2 mW/cm² [$P = <0.0001$, 0.006, and 0.02, respectively, by analysis of variance (ANOVA)].

The ILNvs anatomically appear to receive input from the compound eyes and the HB eyelet. To determine whether the ILNv light response is due to synaptic inputs from external opsin-based photoreceptors, we recorded ILNv in *glass60j* (*gl60j*) mutant flies, which lack all external photoreceptors because of a null mutation in the *eyeless* gene (6). The ILNv response to moderate-intensity white light for *gl60j* flies was 1.37 ± 0.09 ($n = 14$; $P = 0.81$

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versus control); under intense blue light, the response of *gl60j* flies was 1.59 ± 0.1 ($n = 5$, $P = 0.82$ versus control, as tested by ANOVA). Thus, the responses to white and intense blue light do not differ between control and *gl60j* mutant flies (Fig. 1C).

The intact ILNv light response in *gl60j* mutant flies suggests that the blue-light photopigment CRY expressed in the ILNv may underlie the response. *Drosophila* CRY is excited maximally at 450 nm and absorbs wavelengths no longer than 530 nm (17), so the ILNv response to discrete wavelength ranges was tested. The ILNvs significantly increased their firing rate in response to both moderate intensity blue-green light (<550 nm; 1.34 ± 0.04 , $n = 26$, $P < 0.001$ by paired *t* test of lights on versus off) and low-intensity blue-violet light (375 to 450 nm; 1.33 ± 0.02 , $n = 70$, $P < 0.001$), but not to red-orange light (>550 nm; 1.04 ± 0.02 , $n = 23$, $P = 0.18$) (Fig. 1, D and E). The spectral profile of the ILNv light response does not differ when tested in *gl60j* mutant flies (Fig. 1E, FF on/off is 1.37 ± 0.09 , $n = 14$, $P = 0.001$ for white light; 0.94 ± 0.05 , $n = 9$, $P = 0.54$ for orange-red light; 1.21 ± 0.07 , $n = 12$, $P = 0.003$ for blue-green; 1.29 ± 0.05 , $n = 14$, $P < 0.001$ for blue-violet light.) For comparisons for a given wavelength range between control and *gl60j*, *P* values were 0.81, 0.42, 0.27, and 0.64 for white, orange, blue-green, and blue-violet light, respectively. The lack of ILNv responsiveness to wavelengths > 550 nm shows that infrared does not contribute to light-

driven increases in firing frequency. The spectral profile of the ILNv light response matches that of CRY but requires about five orders of magnitude higher light intensity than that required for CRY's known role in resetting the circadian clock via TIM degradation and occur between four to five orders more rapidly than the first biochemical indications of CRY-mediated TIM degradation. Last, unlike CRY-mediated TIM degradation, the ILNv light response is reversible, suggesting that the two CRY-mediated phenomena occur via distinct mechanisms.

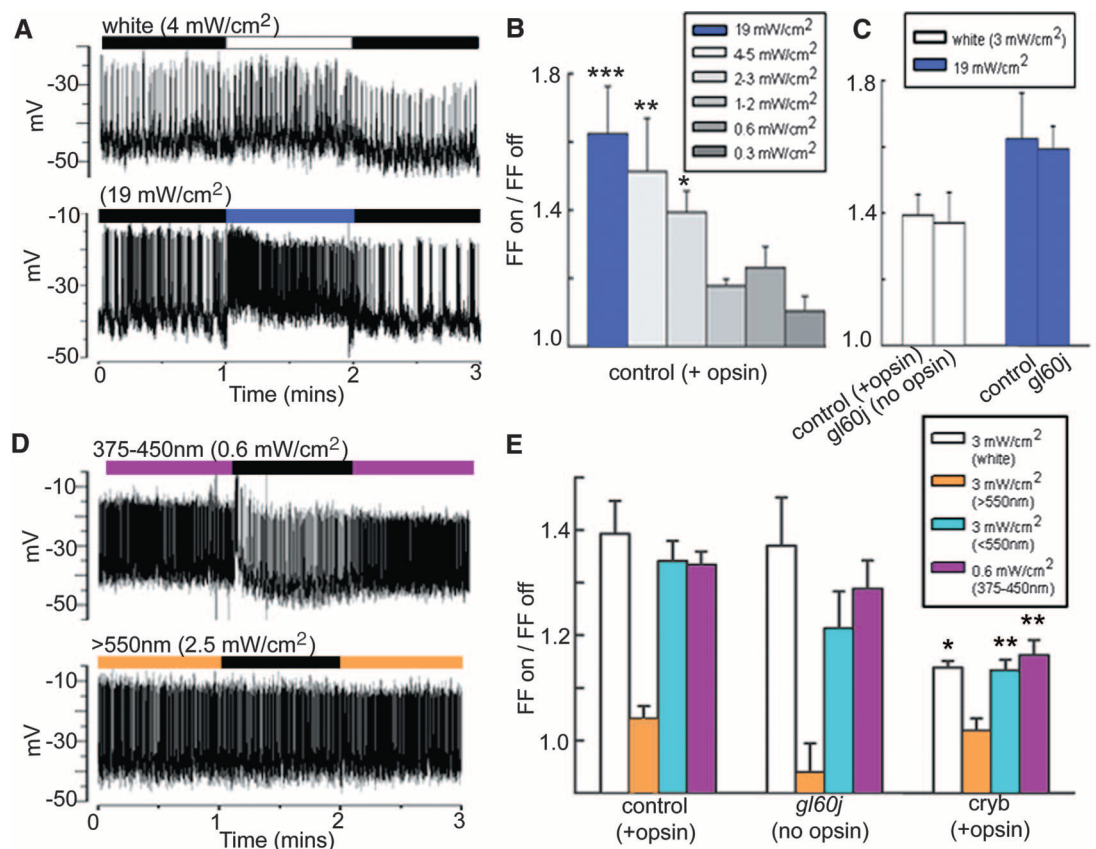
Hypomorphic *cry^b* mutant flies, which exhibit impaired circadian function because of a point mutation in the flavin chromophore binding site, display a weakened ILNv response to white light compared with that of their control counterparts (4). We quantified the light response of *cry^b* ILNvs and tested its spectral properties. Figure 1E (right) shows that, like in control and *gl60j* flies, white, blue-green, and blue-violet wavelengths evoke a significant increase in firing frequency (FF on/off = 1.14 ± 0.01 for white light; 1.13 ± 0.02 for blue-green light; 1.16 ± 0.03 for blue-violet light; $P < 0.01$ by paired *t* test in each case) in *cry^b* mutant flies' ILNvs. However, these *cry^b* light responses are significantly smaller than for their corresponding wavelengths in control flies ($P = 0.02$ for white, $P = 0.005$ for blue-green, and $P = 0.004$ for blue-violet by ANOVA).

To determine whether CRY is necessary for the ILNv light response, we recorded ILNvs from

two CRY-null lines, *cry⁰¹* and *cry⁰²*. Immunocytochemistry confirms baseline native CRY expression in control flies (Fig. 2A), replicating previous results with this CRY antisera (18), and no staining of CRY-positive neurons in either CRY null line (Fig. 2B); however, CRY expression was selectively rescued in the LNvs in both CRY null lines when UAS-CRY was driven by *pdfGAL4* (Fig. 2C). The ILNvs of the *cry⁰¹* and *cry⁰²* null flies exhibited normal spontaneous firing but no light response (Fig. 2, D, top, and E) (*cry⁰¹* response = 1.03 ± 0.04 , $n = 26$, $P = 0.007$ versus control; *cry⁰²* response = 1.04 ± 0.03 , $n = 21$; $P = 0.01$ versus control, ANOVA), indicating that CRY is required for the ILNv light response. The ILNv light response was restored to levels indistinguishable from controls by LNv CRY expression in both *cry⁰¹* (FF on/off = 1.37 ± 0.15 , $n = 17$, $P = 0.99$ versus control) and *cry⁰²* (FF on/off = 1.48 ± 0.10 , $n = 14$, $P = 0.98$ versus control; Fig. 2, D, lower panels, and E) genetic background flies. Although the PDF driver does target the sLNvs in addition to the ILNvs, we have observed light sensitivity in the ILNvs of flies whose sLNvs have been genetically ablated (fig. S1) [also (1)]. Thus, we conclude that the CRY-driven ILNv light response is cell-autonomous and independent of CRY expression in external photoreceptors or other central brain neurons.

To determine whether CRY can optogenetically confer electrophysiological light responsiveness to inherently light-insensitive neurons, we targeted CRY expression to olfactory projec-

Fig. 1. *Drosophila* ILNvs rapidly increase spontaneous action potential firing rate in response to light independent of opsin-based classical photoreceptors. (A) (Top) Response of a representative tonic firing cell to 4 mW/cm² halogen white light. (Bottom) Light response of burst firing cell to 19 mW/cm² blue light. Alternating light/dark cycles denoted by white or blue versus black bars above traces. (B) Firing frequency in light/dark varies according to light intensity. * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.005$; error bars indicate SEM. (C) Genetic ablation of all external opsin-based photoreceptors has no effect on the ILNv light response. (D) Representative recordings of ILNv light response evoked by blue-violet and orange-red light. Alternating light/dark cycles denoted by violet, orange, and black bars. (E) Spectral profiles of light responses of ILNv from control versus *eyeless*-null *gl60j* mutant flies are indistinguishable, but responses to white and blue light of *cry^b* hypomorphs are significantly reduced.



tion neurons with the *GH146-GAL4* driver (fig. S2). This is a rigorous test of (i) CRY's ability to autonomously confer light responsiveness, (ii) a conserved mechanism for coupling CRY light activation to membrane changes in nonclock neurons, and (iii) independence of CRY/TIM interaction. CRY-expressing olfactory neurons increased firing rate recorded in voltage clamp mode in response to intense blue light (Fig. 3A), whereas control CRY-minus olfactory neurons were nonresponsive to white (FF on/off = 1.04 ± 0.02 , $n = 8$), blue-green (0.99 ± 0.04 , $n = 8$), blue-violet (1.01 ± 0.03 , $n = 8$), intense blue (1.04 ± 0.03 , $n = 8$), and orange-red (0.96 ± 0.06 , $n = 8$) light (Fig. 3B, left cluster of bars). The spectral profile of the light response of the

CRY-expressing (*GH146-GAL4/UAS-CRY*) olfactory projection cells is almost identical to CRY-positive ILNvs (Fig. 3B, right) responding to white (FF on/off = 1.14 ± 0.02 , $n = 22$), blue-green (1.27 ± 0.04 , $n = 10$), blue-violet (1.18 ± 0.04 , $n = 18$), and intense blue light (19 mW/cm^2 to 35 mW/cm^2 , 1.47 ± 0.09 , $n = 10$), but not to orange-red light (1.03 ± 0.03 , $n = 10$). The light response of the CRY-expressing olfactory neurons was significantly higher for blue-green ($P < 0.0001$), blue-violet ($P < 0.005$), and intense blue light ($P < 0.0001$, one-way ANOVA) than counterparts measured in control (non-CRY-expressing) cells.

To probe the mechanism of the CRY-mediated light response, we assessed the ability of differ-

ent CRY isoforms expressed in the LNV to rescue the light response in CRY-null flies. The *cry^m* mutant (a nine-amino acid C-terminal truncation of CRY) binds equally well to TIM in light and dark (19), whereas wild-type CRY interaction with TIM is light-dependent. This allowed us to ask whether a light-dependent CRY-TIM interaction is necessary for the acute light response of the ILNvs. The firing rate of CRY^M-expressing ILNV increased significantly in response to white (FF on/off = 1.19 ± 0.05 , $n = 14$) or blue-violet (1.28 ± 0.06 , $n = 12$, $P = 0.20$ versus white light), but not to red-orange light (1.00 ± 0.05 , $n = 9$, $P = 0.005$ versus white light) (Fig. 4, A and B). Significant large light responses were recorded in the ILNvs in *tim*-null flies for moderate-

Fig. 2. The ILNV light response is absent in *cry*-null flies but is functionally rescued by targeted expression of CRY in the LNVs. (A) Native CRY is detected by anti-CRY and colocalizes in the LNV to a *cryGAL4*-driven GFP signal. (B) Anti-CRY signal (red, middle) is absent in LNV (green *pdfGAL4/UAS-dORK-NC1-GFP*, left) in *cry⁰¹* and *cry⁰²* null flies (overlay, right). (C) Verification of anti-CRY signal (red) expressed specifically in LNV (labeled in green, left, *pdfGAL4/UAS-dORK-NC1-GFP*) in *cry⁰¹* and *cry⁰²* null flies that express CRY driven by *pdfGAL4* (overlay, right). (D) Representative recordings of *cry*-null flies (top) and genetic rescue of the ILNV light response in *cry*-null flies (bottom). (E) ILNV FF response evoked by 3 mW/cm^2 white light/dark does not differ between control and LNV specific expression of CRY in *cry⁰¹* and *cry⁰²* null genetic background flies.

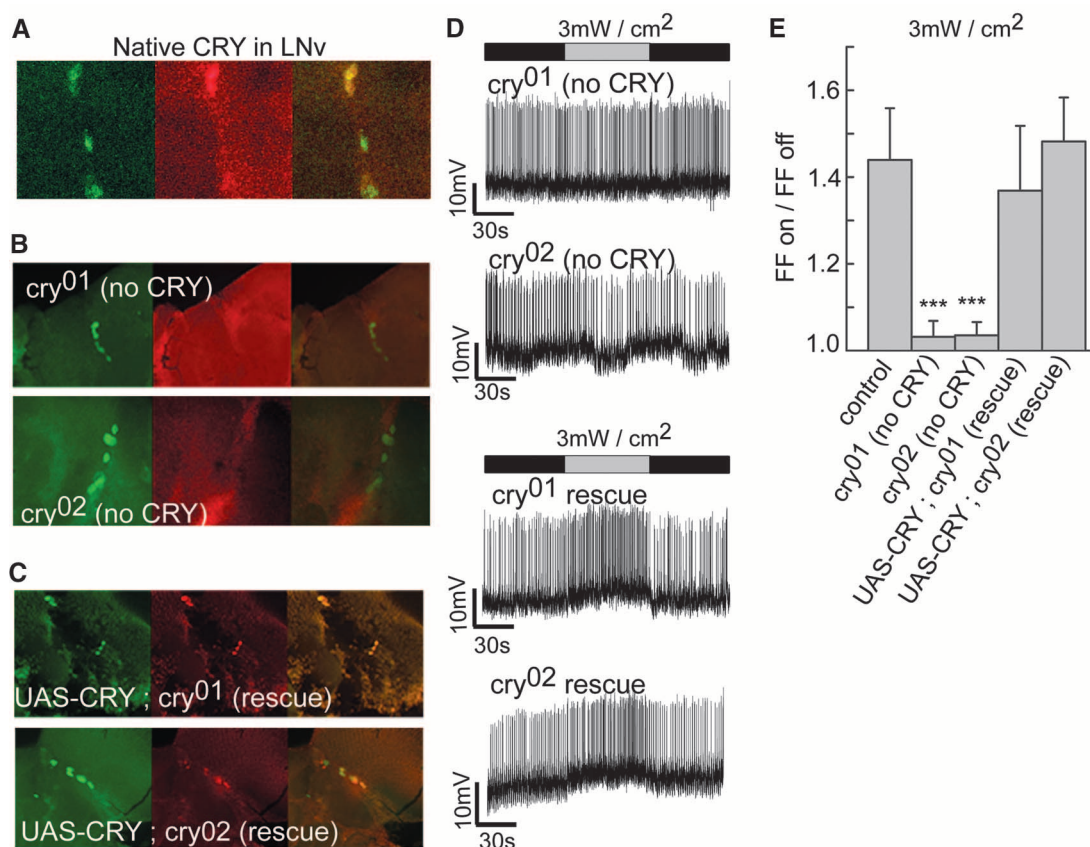
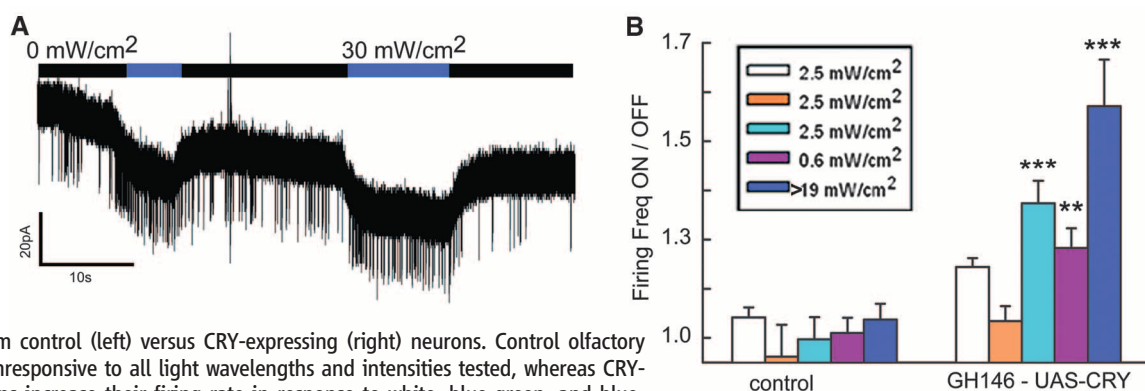


Fig. 3. Ectopic expression of CRY in inherently light-insensitive neurons renders them light-responsive. (A) Representative voltage clamp recording of CRY-expressing olfactory projection neuron shows light response evoked by 30 mW/cm^2 blue light. (B) Spectral profile of light responses of olfactory projection neurons recorded from control (left) versus CRY-expressing (right) neurons. Control olfactory projection neurons are nonresponsive to all light wavelengths and intensities tested, whereas CRY-expressing olfactory neurons increase their firing rate in response to white, blue-green, and blue-violet light but not to orange light.



intensity white (FF on/off = 1.24 ± 0.03 , $n = 14$) and low-intensity blue-violet light (1.25 ± 0.04 , $n = 14$, $P = 0.89$ versus white light), but not for moderate-intensity orange-red light (1.00 ± 0.02 , $n = 14$, $P = 0.01$ versus white light) (Fig. 4C). Thus, TIM interaction is not necessary for the CRY-mediated ILNv light response.

In addition to having the *Drosophila*-like light-responsive CRY1, many insects express a second, vertebrate-like CRY2 that is a potent transcriptional regulator but lacks light sensitivity (20). Because *Drosophila* CRY may also act as a transcriptional regulator (21), we recorded ILNvs expressing either monarch butterfly (*Danaus plexippus*) dpCRY1 or dpCRY2 in the ILNvs of *cry⁰¹* flies. The ILNvs expressing dpCRY1 were electrophysiologically responsive to moderate-intensity white (FF on/off = 1.19 ± 0.05 , $n = 15$) and low-intensity blue-violet (1.16 ± 0.04 , $n = 14$, $P = 0.27$ versus white light) light but not to moderate-intensity orange-red light (1.01 ± 0.05 , $n = 10$, $P = 0.007$ versus white light, ANOVA) (Fig. 4, B and C). In contrast, ILNvs expressing dpCRY2 showed no significant response to white (FF on/off = 1.06 ± 0.04 , $n = 12$), blue-violet (1.02 ± 0.04 , $n = 14$, $P = 0.78$ versus white), or orange-red (1.02 ± 0.06 , $n = 8$, $P = 0.86$ versus white) light (Fig. 4, B and C). Paired *t* tests comparing firing frequency in light versus dark showed no significant response for any wavelength for dpCRY2 ($P = 0.62$ for white, 0.23 for blue-violet, and 0.74 for orange-red light). Thus, the light response requires CRY that is light-sensitive but not transcriptionally active.

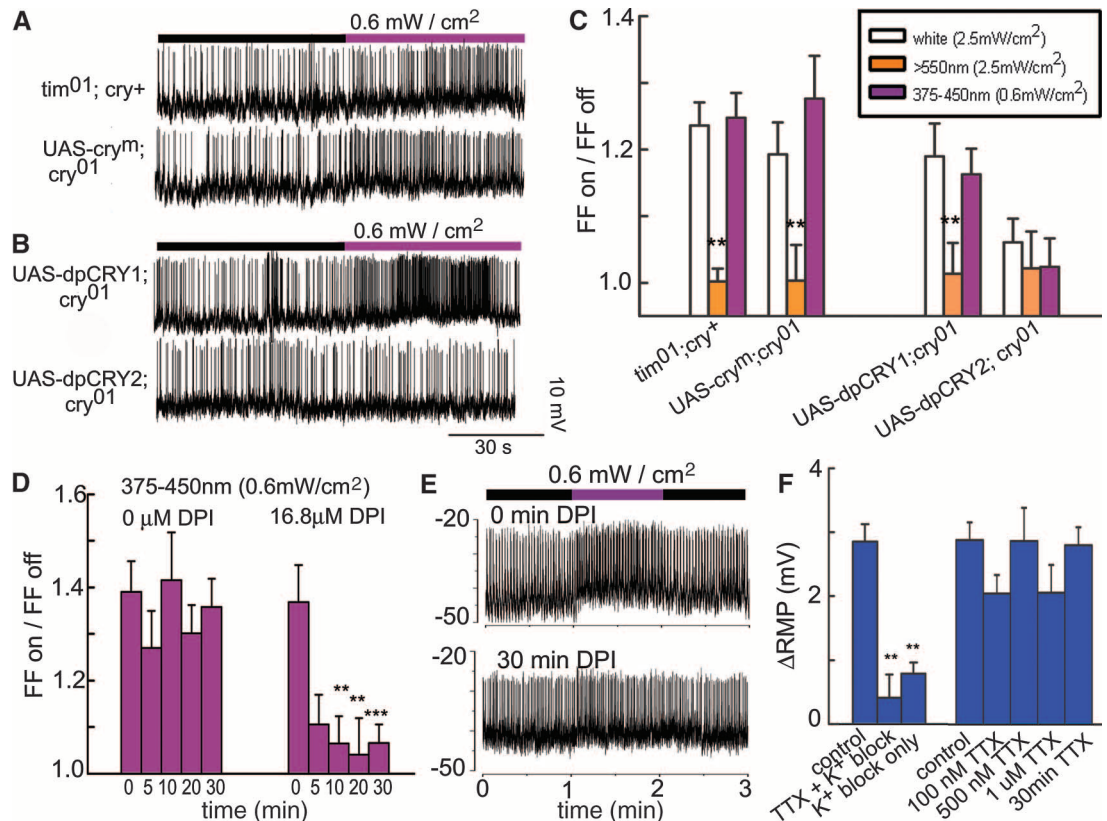
Because a point mutation destabilizes the flavin chromophore binding site in *cry^b* mutants (9), we asked whether acute inhibition of the light-activated flavin redox reaction of CRY blocks the ILNv light response. The ILNv respond to blue-violet light at baseline (Fig. 4, D and E, FF on/off = 1.37 ± 0.08 , $n = 13$). Within 5 min of bath-applied flavin-specific redox inhibitor diphenyleneiodonium chloride (DPI), the response was attenuated (FF on/off = 1.10 ± 0.06 , $n = 5$, $P = 0.16$ versus 5-min sham perfusion). Within 10 min of DPI exposure, blue-violet light no longer evoked a light response (FF on/off = 1.06 ± 0.06 , $n = 10$, $P = 0.0008$ for 10 min; 1.04 ± 0.08 , $n = 11$, $P = 0.01$ for 20 min; 1.06 ± 0.04 , $n = 13$, $P = 0.0008$ for 30 min; all comparisons by ANOVA versus same time in sham perfusion). In contrast, the ILNv light response in vehicle-treated controls was stable over 30 min (FF on/off = 1.39 ± 0.07 and $n = 10$ at zero; 1.27 ± 0.08 , $n = 6$ at 5 min; 1.42 ± 0.10 , $n = 10$ at 10 min; 1.30 ± 0.06 , $n = 10$ at 20 min; and 1.36 ± 0.06 , $n = 11$ at 30 min; $P > 0.31$ all time points compared by ANOVA) (Fig. 4D). Note that DPI does not alter spontaneous baseline firing frequency recorded from the ILNv for up to 30 min, indicating the absence of nonspecific metabolic effects on firing (Fig. 4E).

Light-activation of the flavin chromophore of CRY appears to couple to depolarization of neuronal membrane potential, resulting in increased firing rate. We measured the amplitude of intense blue light-evoked resting membrane potential changes (Δ RMP) in current clamp of opsin-free

gl60j flies (Δ RMP = $2.85 \text{ mV} \pm 0.3$, $n = 45$) then tested the hypothesis that potassium channel modulation underlies coupling of light-activated CRY to membrane depolarization. To visualize light-evoked ILNv membrane potential changes more clearly, we blocked action potentials in these recordings well past saturation (fig. S3) with the voltage-gated sodium channel blocker tetrodotoxin (TTX), and we assessed the resting membrane potential (RMP) response to light. TTX did not significantly reduce these shifts at 100 nM (Δ RMP = 2.03 ± 0.3 , $n = 24$, $P = 0.4$ versus control), 500 nM (2.84 ± 0.5 , $n = 7$, $P = 1.0$ versus control), or 1 μ M (2.04 ± 0.4 , $n = 13$, $P = 0.7$) (Fig. 4F, right). Light-evoked membrane potential changes were, however, significantly decreased by a subsequent application of a voltage-gated and inward rectifier potassium channel blocker cocktail including 10 mM tetraethylammonium (TEA), 2 mM 4-aminopyridine (4-AP), and 2 mM cesium chloride in the presence of 100 nM TTX (Δ RMP = $0.41 \text{ mV} \pm 0.3$, $n = 34$, $P < 0.001$ versus control). We performed time-matched controls with 100 nM TTX only and found that RMP changes did not differ from control (Δ RMP = $2.77 \pm 0.3 \text{ mV}$, $n = 12$, $P = 0.9$). The K-blocker cocktail also significantly disrupted membrane potential shifts in the absence of TTX ($0.78 \text{ mV} \pm 0.2$, $P < 0.001$ versus control, $P = 0.9$ versus K blockers plus TTX). These results suggest that potassium channel modulation couples to the CRY-mediated ILNv light response.

To determine the precise timing of the CRY-mediated light response, we made recordings in ILNv of *gl60j* mutant flies illuminated with a

Fig. 4. The CRY-mediated electrophysiological light response membrane depolarization by potassium channel modulation depends on flavin-specific redox reactions rather than TIM interaction. (A) Representative recordings of ILNv expressing CRY but *tim* null (*tim⁰¹*, top) and *cry⁰¹* with *pdfGAL4*-driven CRY^M (*CRY^M*, bottom). (B) Recordings from ILNvs expressing UAS-driven *D. plexippus* dpCRY1 (top) or dpCRY2 exposed to blue-violet (purple bar) after darkness (black bar). (C) White and violet light evoke significant responses from each genotype except *dpCRY2*; *cry⁰¹*. (D) Treatment with the redox inhibitor DPI rapidly attenuates the light response. (E) Representative recordings of light-evoked responses in vehicle (top) versus 30 min of DPI treatment (bottom) for the same cell. (F) Light-evoked depolarization in ILNv is significantly decreased after treatment with potassium channel blockers TEA, 4-AP, and CsCl in the presence or absence of TTX.



software-triggered blue light source. After ~60 cycles of 4-s blue light pulses from four different ILNvs, records were analyzed in 100-ms bins for spike frequency, 1 s before and after light onset. Although a trend toward more action potentials can be observed during the light pulse, this measurement does not clearly resolve the onset of increased firing in response to light at a millisecond time scale (Fig. 5A). However, application of episodic blue light pulse protocol followed by averaging 120 traces (30 each from four ILNvs) precisely registered by light-on and -off effectively filters the noise from individual records and yields a clear light-evoked RMP response (Fig. 5B). Kinetic analysis reveals that the averaged light-evoked response is best fit with two exponentials with a fast component ($\tau = 105$ ms) and a slower component ($\tau = 1.07$ s). Similarly, the averaged return to the baseline “dark” RMP is also best fit with two exponentials (fast, $\tau = 106$ ms; slow, $\tau = 1.27$ s). Notably, the fast components of the on and off response are nearly identical. The speed of the on response (≈ 100 ms) is within an order of magnitude of that of classical opsin-based phototransduction (22), suggesting that coupling light-activated CRY to depolarization may be diffusion-limited and require intermediate steps. Figure 5C shows individual records that contributed to the averages shown in Fig. 5, either A or B. Although most recordings show an appreciable rise in RMP

(sweeps 2 to 5) within 50 ms after lights on (indicated by the arrow and blue shading), increases in firing frequency were difficult to resolve in time bins of this length. Taken together, the results in Figs. 4 and 5 are consistent with the idea that light-activated CRY couples to membrane depolarization via potassium channel modulation.

The white light intensity levels used to characterize the ILNv electrophysiological light response, although five times higher than intensities necessary to induce CRY-TIM interaction, correspond to natural light levels typically observed in the early to mid-morning on a clear day, consistent with recent findings that the ILNv are light-activated morning arousal neurons (1–4, 23). We asked what percentage of blue light permeates the cuticle and reaches central brain neurons. We found that the average transmittance through the head cuticle, when compared to phosphate-buffered saline mounting buffer alone, was $55.0\% \pm 4.3$ ($n = 7$). Importantly, the transmittance through the eye cuticle is similar at $57.2\% \pm 5.3$ ($n = 7$, $P = 0.74$ by t test).

CRY mediates a rapid electrophysiological light response that is distinct from classical opsin-based phototransduction. However, the present results do not rule out the possibility that light-activated synaptic inputs from external photoreceptors co-modulate ILNv firing rate in intact animals. Dye-filled individual ILNv cells show extensive arbors in the optic lobe and may re-

flect sites for external photoreceptor input (24). All lines of evidence herein indicate that the CRY-mediated electrophysiological light response is mechanistically distinct from the previously described CRY-TIM interaction. Qualitatively, the CRY-mediated electrophysiological light response bears some resemblance to melanopsin-based light activation of retinal ganglion cells (which underlies circadian entrainment in mammals), whereby light activation leads to increased action potential firing rather than graded potentials found typically in image-forming photoreceptors. The CRY-mediated electrophysiological response appears to exhibit a higher light threshold compared with opsin-based light sensing, which may bias its physiological functions to non-image-forming photosensitive cells.

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25. The authors thank P. Hardin (anti-CRY), D. O'Dowd (*gh146GAL4*), J. Hall (*cry⁰¹* and *cry⁰²*), S. Reppert (*UAS-dpCRY1* and *UAS-dpCRY2*), P. Emery (*UAS-cry⁰¹*), and the Bloomington Stock Center for reagents; S. Vasu, M. Digman, and A. Starr for helpful discussion and technical advice; B. Horne for helpful comments on the manuscript and technical assistance; and S. DeGroot and D. Mumford for technical assistance. This work was funded by NIH grant NS046750 to T.C.H. The authors have no competing financial interests.

Supporting Online Material

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Materials and Methods
Figs. S1 to S3
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References

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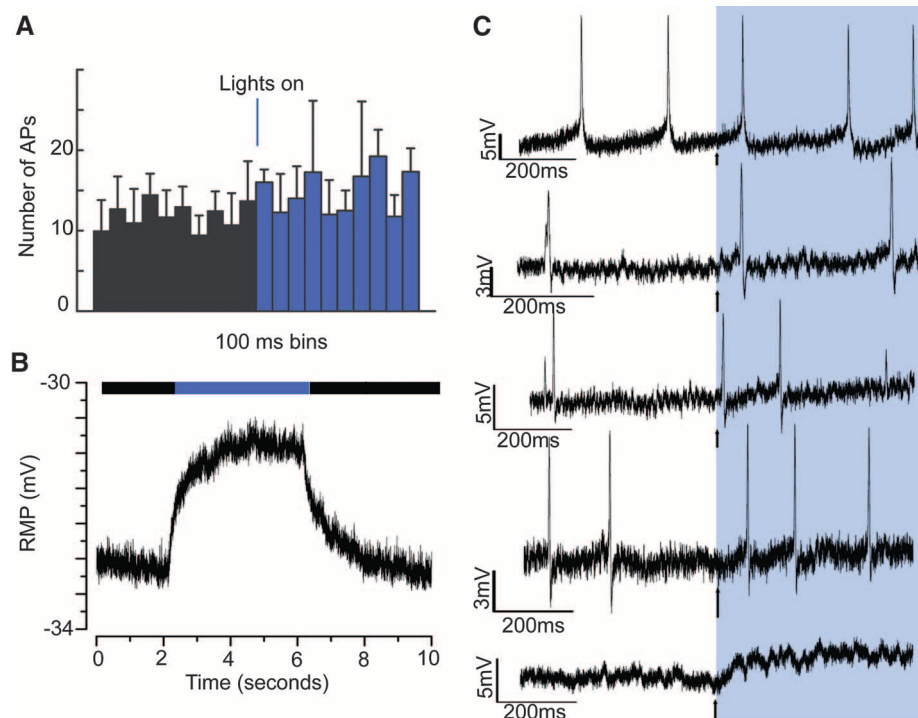


Fig. 5. RMP changes rapidly upon lights on and off. (A) Number of spikes in 100-ms bins shown for 1 s before and after the onset of an intense blue light pulse (~ 35 mW/cm²), averaged for 60 sweeps from four ILNvs. (B) The RMP shows an evoked increase upon triggering of the intense blue light. Trace is an average of 120 sweeps recorded from four ILNvs. The evoked rise and fall were fitted with double exponential functions. (C) Individual records that contributed to the averages depicted in (A) and (B). Four of five traces (bottom four traces) show an appreciable rise in RMP within 100 ms, but the increase in FF for the four cells that are firing is apparent only after several hundred milliseconds.

Rapid and Extensive Surface Changes Near Titan's Equator: Evidence of April Showers

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Although there is evidence that liquids have flowed on the surface at Titan's equator in the past, to date, liquids have only been confirmed on the surface at polar latitudes, and the vast expanses of dunes that dominate Titan's equatorial regions require a predominantly arid climate. We report the detection by Cassini's Imaging Science Subsystem of a large low-latitude cloud system early in Titan's northern spring and extensive surface changes (spanning more than 500,000 square kilometers) in the wake of this storm. The changes are most consistent with widespread methane rainfall reaching the surface, which suggests that the dry channels observed at Titan's low latitudes are carved by seasonal precipitation.

Titan's landforms include high-latitude lakes of liquid hydrocarbons [e.g., (1, 2)] and vast equatorial areas of long-lived longitudinal dunes (3), indicating that low latitudes are primarily arid (4). However, fluvial channels are

observed at all latitudes (5), and the Huygens Probe detected moisture (methane) in the shallow subsurface (6–8) of the cobble-strewn flood plain at ~10°S where it landed (9, 10). To date, Cassini observations span only about one-fourth of a Titan year (2004–2011): late southern summer to early northern spring. Thus, the extent to which the distribution of surface liquids changes over a titanian year (or over longer time scales) is unknown. Does methane rain flood Titan's low-latitude channels during rare seasonal storms, between which the surface dries out (11), or are the channels remnants of an earlier, wetter equatorial climate (12)?

Changes in weather patterns have accompanied Titan's seasons: Storm activity over Titan's south (summer) pole during 2004–2005 (13, 14),

including one observation of possible surface flooding (13), appears to have given way to cloud outbursts at lower latitudes (15, 16). Models predict low-latitude storms around equinox, although insufficient precipitation to accumulate there over the course of a year (11), consistent with the presence of dune fields. Two major low-latitude cloud events have been observed, at ~247°W in April 2008 (15) and at ~320°W on 27 September 2010 (Fig. 1), by Cassini's Imaging Science Subsystem (ISS). In both cases, cloud activity was observed at low latitudes over several weeks (15, 16).

ISS observations in October 2010 (Figs. 1 and 2) of a region east of the cloud outburst [Titan's clouds usually move eastward (16)] revealed differences in surface brightness along the southern boundary of Belet, an extensive dune field. Some of the bright terrain bordering Belet darkened by >10% while adjacent areas remained unchanged (Fig. 2). Although clouds obscured some areas on 14 October, changes had occurred by that time (Fig. 2F). However, in many areas the change has been short-lived: Only some of the darkened area persisted through 29 October (Fig. 2G), and even more territory had reverted by 15 January 2011 (Fig. 2H). A few isolated areas may have brightened relative to their original appearance (Fig. 1E, Fig. 2H, and fig. S1).

The darkening extends ~2000 km east-west and >130 km across. Although changes are more difficult to distinguish in terrain that was originally dark, we have detected differences in some of these areas too. The measured extent of changes that persisted until 29 October is $510,000 \pm 20,000 \text{ km}^2$.

Titan's dark regions consist of hydrocarbons (2, 4, 17, 18), and brighter material is thought to be bright aerosol deposits (18). Cassini synthetic aperture radar (SAR) and Visual and Infrared

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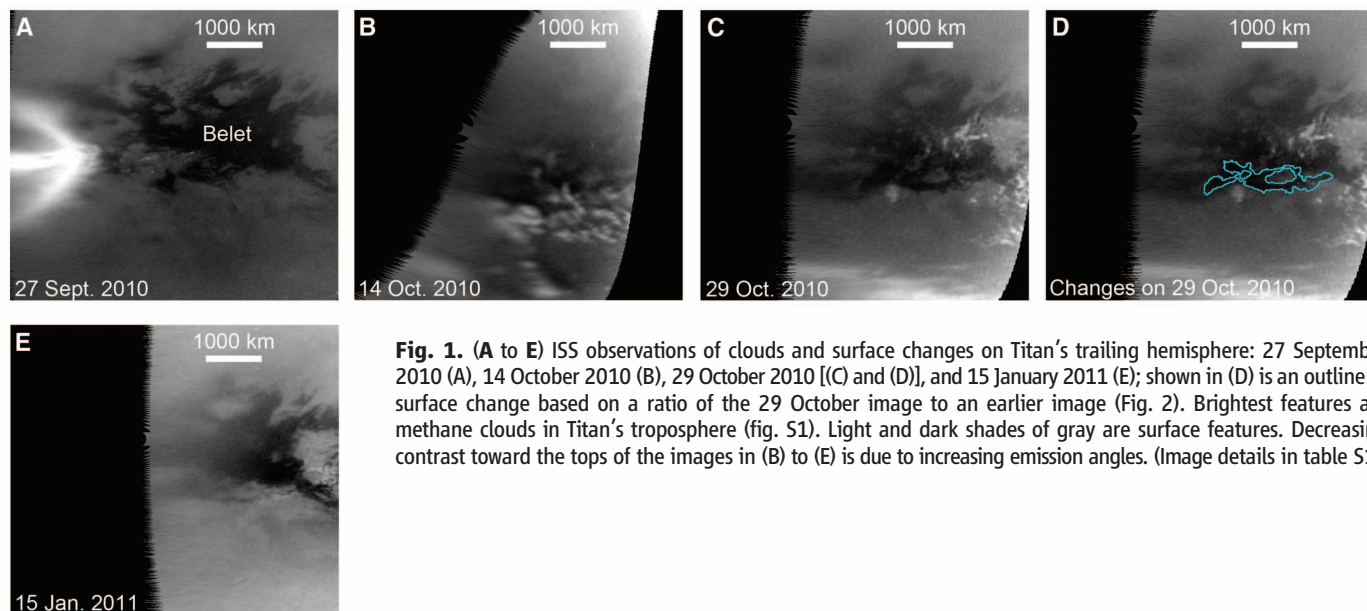


Fig. 1. (A to E) ISS observations of clouds and surface changes on Titan's trailing hemisphere: 27 September 2010 (A), 14 October 2010 (B), 29 October 2010 [(C) and (D)], and 15 January 2011 (E); shown in (D) is an outline of surface change based on a ratio of the 29 October image to an earlier image (Fig. 2). Brightest features are methane clouds in Titan's troposphere (fig. S1). Light and dark shades of gray are surface features. Decreasing contrast toward the tops of the images in (B) to (E) is due to increasing emission angles. (Image details in table S1.)

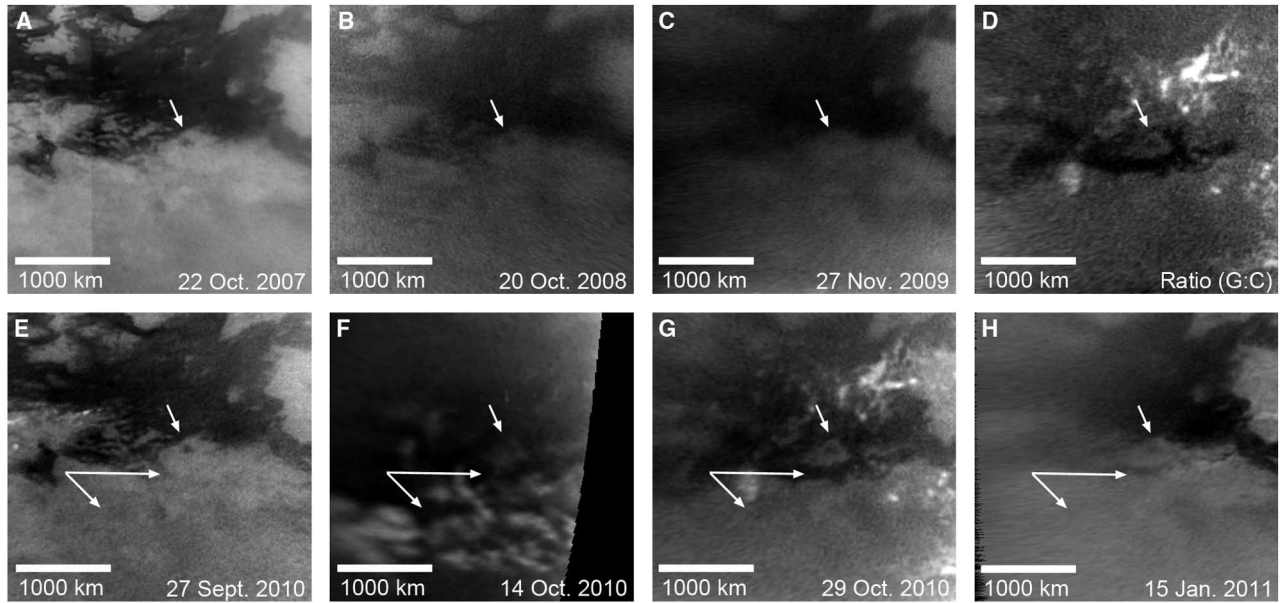


Fig. 2. (A to C and E to H) Sequence of ISS observations. Single arrow indicates isolated unchanged area between Belet and the darkened swath. Double arrow indicates locations of changes that reverted between 14 and 29 October 2010

(lower arrow) and that persisted through October and began to revert by January 2011 (upper arrow). (D) Ratio of observations acquired at similar phase angles on 29 October 2010 (G) and 27 November 2009 (C). (Image details in table S1.)

Fig. 3. (A and B) SAR over September 2010 ISS (A) and August and December 2009 VIMS (B) with red, 5.0 μm ; green, 2.0 μm ; blue, 1.3 μm . SAR from Cassini Titan flybys designated T8, 28 October 2005; T19, 9 October 2006; T21, 12 December 2006; T39, 20 December 2007; T41, 22 February 2008; T49, 21 December 2008; T50, 7 February 2009; T57, 22 June 2009; T61, 25 August 2009; T64, 28 December 2009. (C) Zoom of T49 SAR over ISS. Blue outline indicates area of change as observed on 29 October 2010.

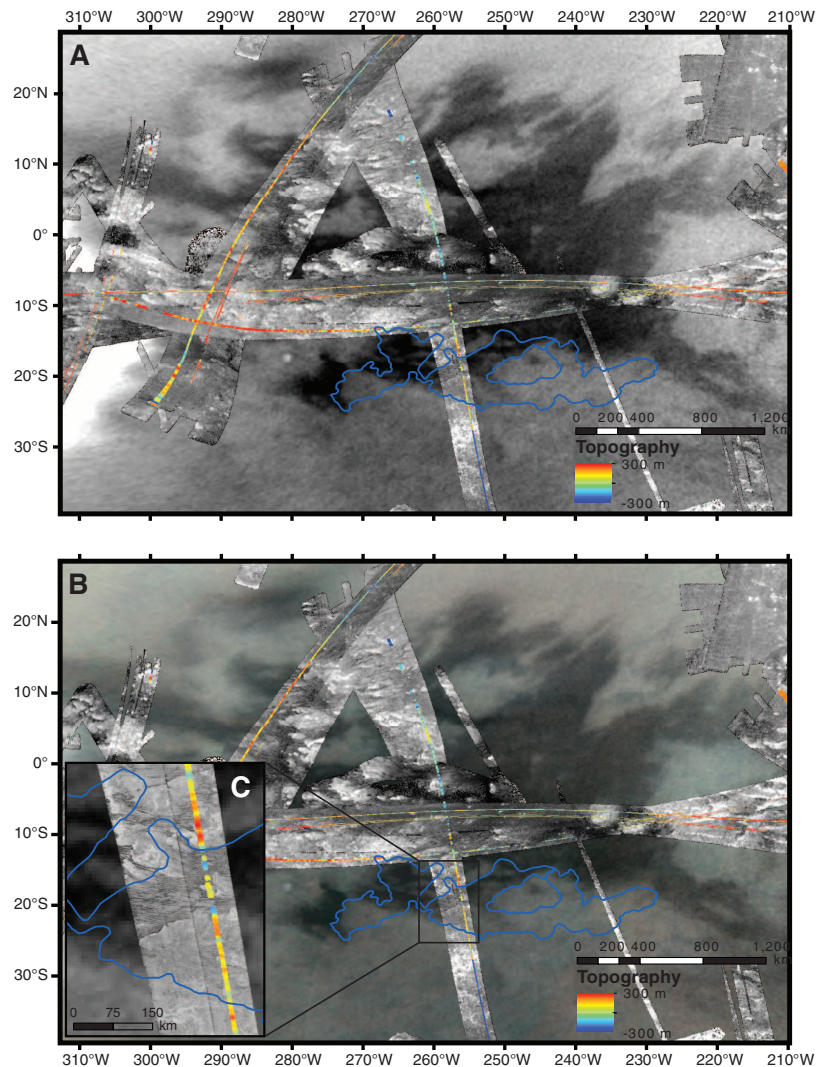


Table 1. ISS observations of Titan's surface and clouds south of Belet. (Image details in table S1.)

Date	Phase angle	Pixel scale (km)	Appearance
13 May 2007	29°	1.9	Normal
*22 Oct 2007	23°	2.7	Normal
Apr–May 2008 (Earth-based)	4.9°	130	Large cloud, 29°S, 247°W (15)
*20 Oct 2008	89°	5.3	Normal; similar (high) phase angle to 29 Oct 2010
*27 Nov 2009	89°	7.2	Normal; similar (high) phase angle to 29 Oct 2010
13 Sep 2010	70°	15	Normal
*27 Sep 2010	44°	7.7	Large arrow-shaped cloud to west (~12°S, 300°–340°W); surface appeared normal coincident with cloud
*14 Oct 2010	113°	12	New dark territory south of Belet
*29 Oct 2010	88°	11	Decrease in darkened area
15 Jan 2011 (03:15 UTC)	78°	5.0	Continued decrease in darkened area
*15 Jan 2011 (18:00 UTC)	66°	6.0	Continued decrease in darkened area

*Image in Fig. 2.

Mapping Spectrometer (VIMS) observations of this region (Fig. 3) confirm the presence of dunes in areas originally seen by ISS to be dark. The boundaries of the changed region do not correlate with preexisting albedo boundaries in ISS and VIMS observations or with obvious morphologic or topographic boundaries in the SAR data (Figs. 2 and 3).

We can rule out observational effects and clouds, thereby establishing that the differences represent changes on Titan's surface. ISS has observed this region several times since May 2007 (Table 1): Its appearance was consistent before October 2010, regardless of phase angle. Images acquired in October 2008 and November 2009 at phase angles and resolutions similar to those in October 2010 show nothing unusual when compared to images acquired at lower phase angles from October 2007 and September 2010 (Fig. 2). Areas with consistent borders from 14 to 29 October exhibit a level of constancy not observed in Titan's clouds (16), and even low-lying clouds (17) and features identified as fog (19) are bright at the ISS wavelength used for Titan (938 nm). Cloud shadows are also unlikely: Titan's substantial atmospheric scattering (10) diffuses shadows, whereas the changed areas are distinct at pixel scales of ≤ 12 km.

Methane precipitation could affect a huge area over a short period of time, explaining the rapid appearance (and disappearance) of the changes and their extensive and nonuniform nature. The cloud observed on 27 September was more than 1000 km in extent. Surface brightness could change by flooding or wetting, which renders materials darker by changing their optical properties (20, 21). The degree of darkening is comparable to surface liquids seen elsewhere by ISS (13). In the case of flooding, areas of change should correlate with low-lying areas. However, the narrow strip of SAR topography that crosses the darkened area (Fig. 3) does not demonstrate such a relationship, nor are there any obvious correlations between the new boundaries and morphologic features in the SAR data. Furthermore, the observations would require

standing liquid over an area larger than Kraken Mare, Titan's largest sea. Both of these issues are resolved if, at least in some areas, the darkening is caused merely by surface wetting: Much less precipitation is necessary, and the observed pattern results from variations in precipitation and potentially the nature of the surface. Wetting of fine aerosol particles (22) could be part of the unknown process by which such material is cemented together to form particles large enough to undergo saltation, required for dune formation. Precipitation can also explain the rebrightening observed later in some places as different areas drain (by overland flow or infiltration) or dry at different rates. In an unsaturated permeable medium, vertical infiltration rates will be high [>20 mm/week (23)]. Evaporation rates of 20 mm/week have been documented at Titan's poles (24), and equatorial rates of >1 mm/week are predicted (11). Small areas that might have brightened relative to their original appearance are stationary compared to typical clouds (fig. S2), so they could be bright surfaces [potentially water ice (17)] cleaned by runoff or persistent low-altitude clouds or fog.

Another hypothesis is aeolian modification, perhaps a result of high surface winds accompanying the storm, redistributing dark material or removing bright mantling material (18) to reveal a dark substrate. SAR and VIMS data (Fig. 3) demonstrate the existence of dark dune material in the vicinity of the observed changes. However, assuming sufficient source material, the question is whether winds could transport it hundreds of kilometers over such a short time. A conservative estimate requires a mass flux of 0.12 kg/ms, corresponding to a free-stream wind speed of 2.2 m/s (25). According to large-scale general circulation models (26), sustained winds of such speeds are highly unlikely. Storm-generated downdrafts and gravity currents could enhance surface winds, but at issue is whether they could persist consistently for several days. A critical complication for an aeolian hypothesis is the need for multiple events to explain areas reverting to their previous appearance over time.

Volcanism is another mechanism for rapid large-scale surface changes. Flows would require control by preexisting structures and prohibitively fast deposition of extreme amounts of dark material; terrestrial flood volcanism takes thousands of years to cover comparable areas (27). Explosive cryovolcanism, perhaps more consistent with the time scale and extent of the changes, is not expected on Titan (28).

The most likely explanation for the formation of the low-latitude clouds is a seasonal change in weather patterns encouraging development of convective cloud complexes, perhaps associated with the equatorial crossing of a titanian inter-tropical convergence zone (11, 16, 26). Other possibilities include topographic features generating orographic uplift or cryovolcanic outgassing of methane triggering cloud formation (29). The equatorial lower atmosphere is too dry to support free (unforced) moist convection (12, 30, 31), but a source of methane gas at the surface would increase the relative humidity and thus the potential for convective outbursts. Intriguingly, the only other low-latitude cloud outburst of this scale occurred at similar longitudes (15). However, no cryovolcanic features have been identified in this area, and clouds do not appear to occur here preferentially (16).

Precipitation from a large methane storm over Titan's arid low latitudes, as predicted near equinox by atmospheric models (11), best explains the observed surface changes. Infrequent events would not prevent long-term development and preservation of the dune fields. A few meters of dune erosion, which could be repaired between equinoxes, would not be visible at scales smaller than the SAR resolution of a few hundred meters. Occasional storms are sufficient to form the observed channels (32, 33), and, although the dune fields demonstrate that these latitudes are predominantly dry, they do not preclude occasional precipitation; many terrestrial drylands are geomorphologically dominated by fluvial activity.

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Supporting Online Material

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Figs. S1 and S2

Table S1

References

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Isotopic Evidence of Cr Partitioning into Earth's Core

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The distribution of chemical elements in primitive meteorites (chondrites), as building blocks of terrestrial planets, provides insight into the formation and early differentiation of Earth. The processes that resulted in the depletion of some elements [such as chromium (Cr)] in the bulk silicate Earth relative to chondrites, however, remain debated between leading candidate causes: volatility versus core partitioning. We show through high-precision measurements of Cr stable isotopes in a range of meteorites, which deviate by up to ~0.4 per mil from those of the bulk silicate Earth, that Cr depletion resulted from its partitioning into Earth's core, with a preferential enrichment in light isotopes. Ab initio calculations suggest that the isotopic signature was established at mid-mantle magma ocean depth as Earth accreted planetary embryos and progressively became more oxidized.

Determining the chemical composition of Earth's core provides key constraints on the physicochemical conditions at the time of the planet's formation. Because primitive meteorites are believed to be similar in composition to the material from which Earth accreted (1–4), they provide a good proxy for the undifferentiated bulk Earth composition that eventually differentiated to form the metallic core and silicate mantle. These estimations are most accurate for refractory elements (such as Ca and Al) that did not fractionate by volatilization before or during Earth's accretion. The abundances of the moderately volatile elements in Earth's core are therefore poorly constrained because of difficulties in choosing meteorite samples that represent the bulk Earth (1–4).

Experiments suggest that Cr could fractionate into the core under conditions prevailing in Earth's lower mantle or at the base of a magma

ocean (2, 5–8). It has been shown that the partitioning behavior of Cr is more sensitive to temperature (6, 7) and oxygen fugacity (f_{O_2}) (2, 5, 8) than to pressure (6, 7). Its depletion in the silicate Earth in comparison to chondrites suggests that the Cr could have been partitioned into Earth's core (1–8). However, Cr is also a moderately volatile element (1, 4, 9), and its depletion in the silicate Earth in comparison to bulk chondrites may reflect its volatility (10, 11).

Here we report high-precision stable isotopic compositions of Cr in meteorites to understand the origin of the depletion of Cr in the silicate Earth. It is now possible to measure variations in the stable isotope composition of Cr with high precision and accuracy (12–14). We analyzed the bulk Cr isotopic composition of seven carbonaceous chondrites from the different major groups: Orgueil (CI1), Dar al Gani 749 (CO3.1), Ningqiang (CK3), Vigarano (CV3), Lance (CO3.4), Cold Bokkeveld (CM2), and Murchison (CM2); five ordinary chondrites: Nadiabondi (H4), Forest City (H5), Ausson (L5), Tuxtuac (LL5), and Dimmit (H3.7); one enstatite chondrite: Sahara 97103 (EH3); and six single chondrules from Chainpur (LL3.4) (15) (table S3). The full range of Cr isotope fractionation per atomic mass unit ($\delta\text{Cr}/\text{amu}$) (16) in the chondrites (bulk rock and individual chondrules) is ~0.40 per mil (‰)/amu (Fig. 1).

The condensation/evaporation processes operating in the early solar system may have induced isotopic fractionations of Cr with a loss of light isotopes. The Chainpur chondrules with heavy Cr isotope enrichment appear to show such an effect (Fig. 1). If true, such processes should also affect other elements; in particular, those elements more volatile than Cr. Both Zn and Cu are more volatile than Cr [the condensation temperature (T_c) of Zn = 726 K and $T_c(\text{Cu})$ = 1037 K, versus $T_c(\text{Cr})$ = 1296 K (9)]. In addition, Zn isotopes have been shown to be fractionated during evaporation processes (17–19). However, both Cu (20) and Zn (21) show reverse volatility trends, opposite to Cr (13). The systematics are most pronounced in carbonaceous chondrites. Figure 2, A and B, show that δCr is anticorrelated with δCu , and δZn , respectively. Moreover, δCu and δZn are negatively correlated with refractory/volatile elemental ratios [Mg/Cu , Mg/Zn , $T_c(\text{Mg})$ = 1336 K (9)] (Fig. 2, D and E), whereas δCr is positively correlated with Mg/Cr (Fig. 2F). Most important, the fact that δCr , δZn , and δCu all correlate with $\Delta^{17}\text{O}$ (Fig. 2C) (13, 20–21), a mass-independent fractionation tracer (22), suggests large-scale two-reservoir mixing in the early solar nebula, with one component enriched in light isotopes of Cr and heavy isotopes of Zn and Cu and high $\Delta^{17}\text{O}$, and a second component enriched in heavy isotopes of Cr and light isotopes of Zn and Cu and low $\Delta^{17}\text{O}$. This contrasting behavior of Cr on one hand, and of Cu and Zn on the other hand, and their correlations with $\Delta^{17}\text{O}$, collectively argue against isotopic fractionation by volatilization and are instead consistent with the conclusions of Luck *et al.* (22).

Recently, Schoenberg *et al.* (14) showed that terrestrial igneous silicates, including mantle xenoliths, ultramafic cumulates, and oceanic as well as continental basalts, are isotopically homogeneous in Cr and give an average $\delta\text{Cr}/\text{amu}$ = $-0.12 \pm 0.10\text{‰}$ (2 SD) ($\pm 0.02\text{‰}$, 2 SE) for the bulk silicate Earth relative to the SRM 979 Cr standard. Therefore, the silicate Earth is enriched in heavy isotopes of Cr relative to chondrites (Fig. 1). Based on a mass balance between the silicate Earth and the chondrites, the core may control the

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Fig. 1. $\delta^{53/52}\text{Cr}$ versus $\delta^{50/52}\text{Cr}/(-2)$ in chondrites. Error bars are 2 SE [standard error of the mean, $2\text{ SD}/(n)^{1/2}$]. The numbers of repeat measurements n are shown in table S3 (15). For measurements performed less than three times, 2 SE internal errors are reported. The gray box represents the full range of δCr for terrestrial igneous silicates (14) ($\delta\text{Cr}/\text{amu} = -0.12 \pm 0.10\text{‰}$, 2 SD). With the exception of individual chondrules from Chainpur and one ordinary chondrite (Forest City), all bulk chondrites from this study are distinct from terrestrial igneous silicates in $\delta\text{Cr}/\text{amu}$. The inset at lower right shows the normalized probability density distribution plot of terrestrial igneous silicates [red curve (14)] compared to the various chondrite group data from this study (pink curve, excluding chondrule data from Chainpur).

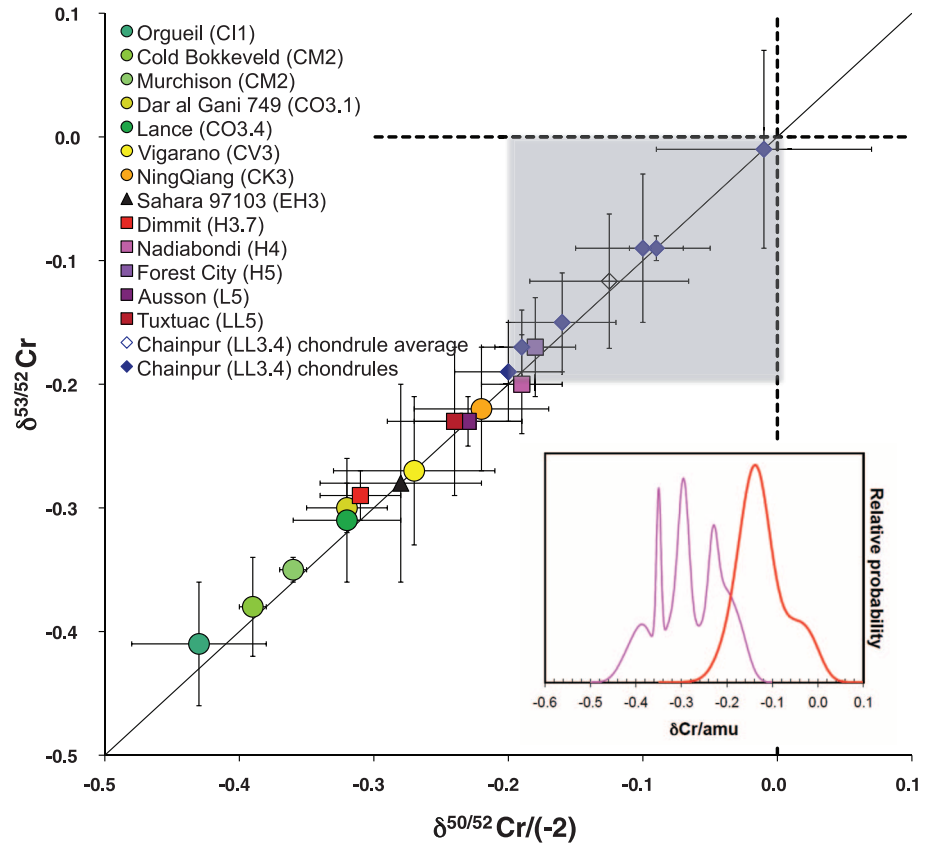


Fig. 2. Reverse volatility trend shown by anticorrelations between $\delta\text{Cr}/\text{amu}$ and $\delta\text{Cu}/\text{amu}$ (A) and $\delta\text{Cr}/\text{amu}$ and $\delta\text{Zn}/\text{amu}$ (B), as well as by anticorrelations of $\delta\text{Cu}/\text{amu}$ and $\delta\text{Zn}/\text{amu}$ with refractory/volatile elemental ratios (Mg/Cu and Mg/Zn) (D and E), in contrast to positive correlation between $\delta\text{Cr}/\text{amu}$ and Mg/Cr (F). Mass-dependent isotope fractionations of Cr, as well as Cu and Zn, correlate with $\Delta^{17}\text{O}$ (C). $\Delta^{17}\text{O}$ is a vertical deviation from the terrestrial fractionation line in three oxygen isotope plots, as a measure of mass-independent fractionation (22). The origin of $\Delta^{17}\text{O}$ variation in the solar system is most likely due to nonequilibrium photochemical dissociation of CO in the early solar nebula (32–34), although $\Delta^{17}\text{O}$ is also known to be correlated with nuclear anomalies of ^{54}Cr (35, 36), a nuclide of supernova origin (37). Data sources are as follows: Cr isotope data, (13, 15) (table S3); Cu and Zn elemental and isotopic data, (20, 21); $\Delta^{17}\text{O}$, (22); and Mg and Cr elemental data, (38–40).

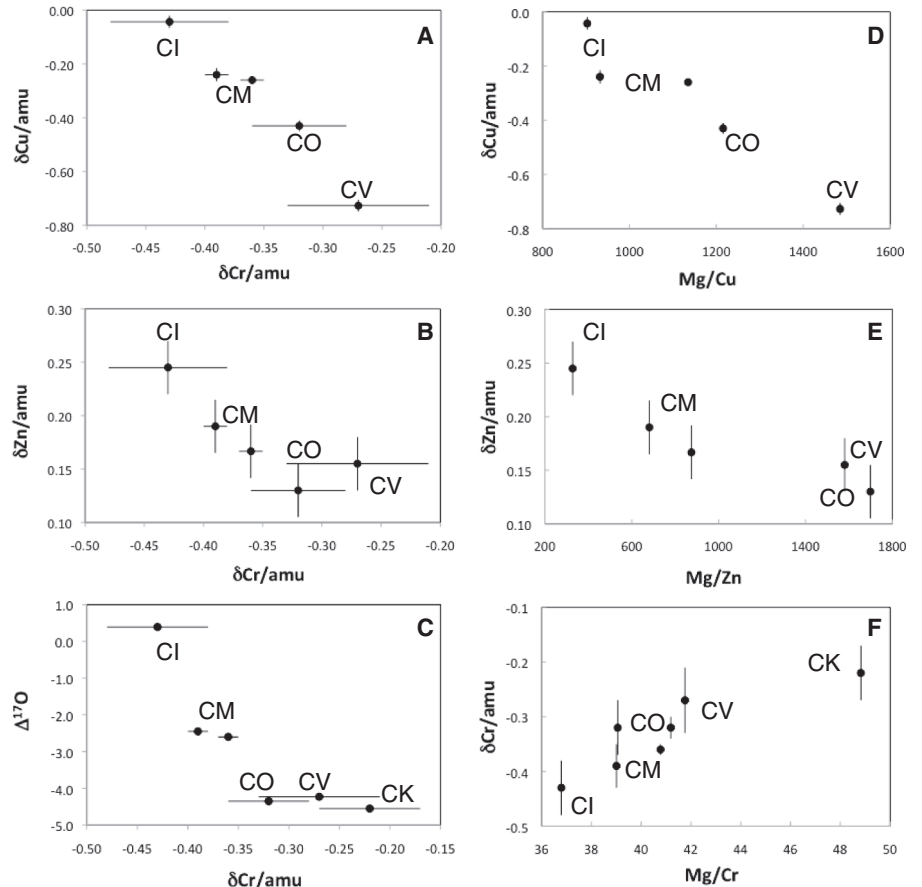


Fig. 3. Cr isotopic composition in the BSE, the bulk Earth, and Earth's core as a function of the percentage of Cr represented by the reservoir (38% of the Earth Cr is in the BSE and 62% in Earth's core). The isotopic composition of the bulk Earth is assumed to be similar to that of bulk ordinary chondrites (model 1) or bulk carbonaceous chondrites (model 2). Data sources are as follows: (1, 3, 14, 15) and table S3.

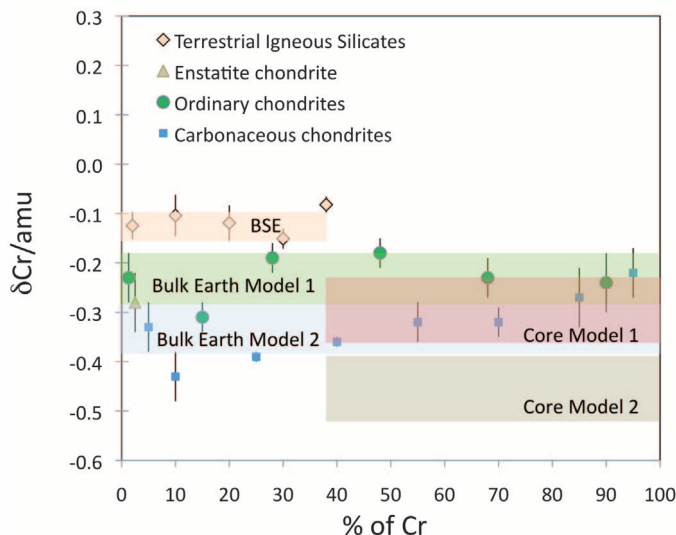
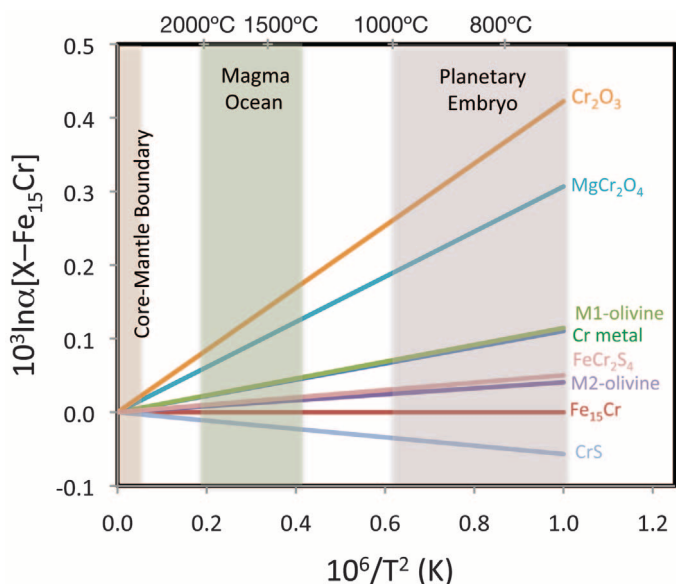


Fig. 4. Calculated equilibrium Cr isotope fractionation ($10^3 \ln \alpha \approx \Delta \delta \text{Cr/amu}$) of eskolaite (Cr_2O_3), magnesiochromite (MgCr_2O_4), olivines, pure Cr metal, daubreelite (FeCr_2S_4), and CrS relative to Fe_{15}Cr as a function of temperature relevant to core formation.



major fraction (60 to 65%) of the total Cr budget for Earth (1, 3). Accordingly, the observed Cr isotopic effect due to core partitioning is far more pronounced than the Si isotopic differences between the bulk silicate Earth (BSE) and the carbonaceous chondrites (CHUR) [$\Delta^{30}\text{Si}_{\text{BSE-CHUR}} = 0.08 \pm 0.04\text{‰}$ (1 SD) (23) or $\Delta^{30}\text{Si}_{\text{BSE-CHUR}} = 0.035 \pm 0.035\text{‰}$ (24)], which translates into less than 1.67% of Si in the core (24).

The Cr isotopic composition of Earth's core can be estimated by using a simple mass balance

$$\delta \text{Cr}_C = \frac{\delta \text{Cr}_E - (1 - f_C) \times \delta \text{Cr}_M}{f_C} \quad (1)$$

where the subscripts C, E, and M represent the core, the bulk Earth, and the silicate mantle respectively, and f_C is the fraction of Cr in Earth's core [estimated to be 0.62 from (1, 3)]. The Cr isotope composition of Earth's core varies with assumptions regarding the bulk Earth composition (Fig. 3). However, in all of the models

considered, the Cr isotope composition of the core is clearly different than that of the BSE, with an isotopic enrichment in light isotopes of up to 0.51‰ per mass unit.

The direction and magnitude of Cr isotopic fractionation in the silicate Earth induced by the partial loss of Cr into the metallic core can be roughly estimated using electronic structure models of representative Cr-bearing phases. During planetary differentiation, Cr (0) (metal → core), Cr (II) (silicate → BSE), and Cr (III) (silicate/oxides → BSE/core) are expected to dominate speciation chemistry. To sample this range of crystal and melt chemistry, we constructed electronic structure models of several crystals, including Cr-metal, an Fe-rich intermetallic crystal (Fe_{15}Cr), forsteritic olivines substituted with one Cr^{2+} atom per unit cell ($\text{Cr}_{0.25}\text{Mg}_{1.75}\text{SiO}_4$), and Cr^{3+} -bearing magnesiochromite (MgCr_2O_4) and eskolaite (Cr_2O_3) (25). Sulfide crystals, including CrS and daubreelite (FeCr_2S_4), were also modeled to investigate possible effects of Cr-S asso-

ciation in sulfide and metallic melts (15). We assume that liquid phases are broadly analogous to crystals with Cr in the same oxidation state, because this is known to be a primary determinant of equilibrium isotope fractionation (25).

The modeled Cr isotopic fractionations ($10^3 \ln \alpha$) vary substantially between the different Cr species and the Fe-rich intermetallic crystal (Fe_{15}Cr) as a function of temperature (Fig. 4). These models suggest that equilibrium partitioning of Cr between a low- $f\text{O}_2$, Cr^{2+} -dominated silicate liquid, sulfide species (CrS and FeCr_2S_4), and metal (Fe_{15}Cr) will not impart a strong isotopic signature to the silicate reservoir unless a dominant fraction of the total Cr mass is fractionally distilled into the metal. At core-mantle boundary temperature, the Cr isotope fractionations are less than 0.02‰ for all species considered. Batch removal at relatively low temperatures (~1500 K) should leave the silicate residue $\delta \text{Cr/amu}$ within 0.1‰ of the bulk planetary composition. The site preference of Cr^{2+} (M1 versus M2) apparently has only a slight effect on isotope fractionation. For a given partitioning of Cr between silicate and core, larger signatures are likely if the silicate has substantial Cr^{3+} , particularly if hosted in oxide (Cr_2O_3 or magnesiochromite MgCr_2O_4) or silicate species, but this appears most likely to occur at relatively high $f\text{O}_2$, possibly limiting the amount of Cr lost to the core and thus isotopic and compositional signatures in the residual silicate mantle. Relatively low temperature and more-oxidizing conditions could be found if the metal/silicate differentiation occurred in planetary embryos (~Moon- to Mars-sized) and as these embryos accreted to form progressively deeper magma oceans on the growing Earth (Fig. 4) (26, 27). Recent dynamical models showed that metal/silicate differentiation must have occurred in small planetary bodies (Moon- to Mars-sized) at fairly low temperature (<1500 K) before Earth's accretion (28, 29). Our conclusion is in good agreement with the experimental partitioning data for Cr and other elements at relevant pressures and temperatures and $f\text{O}_2$ (5, 8, 26, 27), and the modeling of ^{182}Hf - ^{182}W isotope systematics for Earth's core formation (30, 31).

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Direct Observation of Continuous Electric Dipole Rotation in Flux-Closure Domains in Ferroelectric $\text{Pb}(\text{Zr,Ti})\text{O}_3$

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Low-dimensional ferroelectric structures are a promising basis for the next generation of ultrahigh-density nonvolatile memory devices. Depolarization fields, created by incompletely compensated charges at the surfaces and interfaces, depress the polarization of such structures. Theory suggests that under conditions of uncompensated surface charges, local dipoles can organize in flux-closure structures in thin films and vortex structures in nano-sized ferroelectrics, reducing depolarization fields. However, the continuous rotation of the dipoles required in vortex structures and the behavior of unit cell dipoles in flux-closure structures have never been experimentally established. By aberration-corrected transmission electron microscopy, we obtained experimental evidence for continuous rotation of the dipoles closing the flux of 180° domains in a ferroelectric perovskite thin film.

Ferroelectric thin films have demonstrated great potential for microelectronics applications (1–5). In ferroelectric random access memories, information is stored and read by switching and detecting the polarization orientation in the ferroelectric material. Microscopically, polarization switching is realized through the motion of domain walls driven by an external electric field. Phenomenologically, domain arrangements are characterized by the angle between polarization directions in adjacent domains. Stripes of 180° domains have been reported for thin films of PbTiO_3 on SrTiO_3 (6, 7). In epitaxial thin-film systems, the domain pattern is

determined by the growth conditions and by the mechanical interface strain and the electric boundary conditions related to the depolarization field at surfaces or interfaces. Ideally, two such boundary conditions can be distinguished according to whether the surface or interface charges are perfectly screened (e.g., by conducting electrodes) or unscreened (vacuum or insulating surface layers). In addition, the effect of anisotropic electrostriction must be considered. It imposes a substantial energy cost for rotating the polarization away from the normal symmetry-allowed lattice directions. Theoretical studies on $\text{Pb}(\text{Zr}_{1-x}\text{Ti}_x)\text{O}_3$ have revealed a variety of different domain patterns depending on the competition between these factors. Nonetheless, theory predicts as a general feature the formation of a stripe-type pattern of Landau-Lifshitz polarization with 90° flux-closure structures at surfaces or interfaces, whereby the strength of the de-

polarization field is reduced (8–11). Flux-closure structures in ferroelectric materials are currently a subject of intensive interest. Recent experiments reported their existence at the junctions of multiple 71° or 90° domains (12, 13). Flux-closure structures, which were predicted by theory in ferroelectric film systems with 180° stripe domains, have not yet been corroborated by experiment.

Theoretical investigations have shown that in very small ferroelectric structures a few nanometers in size, the local dipoles should organize in vortices rather than in domains with uniform polarization because of the large depolarization fields (14, 15). Experimental results have been reported for $\text{Pb}(\text{Zr}_{0.4}\text{Ti}_{0.6})\text{O}_3$, $\text{Pb}(\text{Zr}_{0.3}\text{Ti}_{0.7})\text{O}_3$, BaTiO_3 nanodots, and fine-grained polycrystalline samples; these have been interpreted in terms of vortex structures (16–18). However, the spatial resolution of these studies was too low to provide direct evidence for the formation of a vortex phase. Recently, Nelson *et al.* reported on observations by aberration-corrected scanning transmission electron microscopy (STEM) of vortex-like nanodomain arrays at 109° domain walls at the interface in BiFeO_3 films grown by molecular beam epitaxy on TbScO_3 substrates (19). A vertical 109° domain wall splits into a mirrored pair of inclined 180° domain walls and a 109° wall of opposite sense. In this way a vortex domain structure is formed, with the polarization rotating about the intersection of two 109° and two 180° domain walls. This complex domain wall arrangement, together with the interface epitaxy constraints, leads to a local broadening of the walls, which makes the polarization rotation quasi-continuous. Although insufficient resolution prevents the accompanying phase-field simulations from reproducing this quasi-continuous rotation, they show that for this particular material combination, such a multidomain arrange-

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ment corresponds to a low-energy situation that minimizes depolarization.

The observations described below, in addition to giving evidence of the existence of flux-closure structures at a 180° domain wall, represent a direct experimental proof of con-

tinuous polarization rotation on the atomic scale. Using atomic-resolution aberration-corrected TEM, we studied domain structures of the epitaxial thin-film system $\text{PbZr}_{0.2}\text{Ti}_{0.8}\text{O}_3$ (henceforth PZT) on SrTiO_3 (STO) (20). We made use of the negative spherical aberration imag-

ing (NCSI) technique, which allows not only location of all the atomic species but also measurement of their positions, unit cell by unit cell, with a precision of a few picometers (21–24). On this basis we calculated the relative ion displacement and the electric dipole moment for the individual unit cells. The resulting map of dipole moments depicts the atomic morphology of domains and domain walls. In this way we identify a near-interface region within the PZT film where the local dipoles rotate continuously, forming a flux-closure structure connecting two 180° domains.

Figure 1 shows an atomic-resolution image of an area including the interface between the PZT layer and the STO substrate. The arrangement of the film layers is schematically shown in fig. S1. In Fig. 1 the structure (one projected unit cell) of PZT is indicated schematically in two regions (positions of Zr-Ti, PbO-SrO, and O atomic columns, seen end-on, are indicated by red, yellow, and blue circles, respectively). Because of the particular imaging conditions, dynamic electron scattering yields a sharp bright contrast (25) in the ~ 11 -nm-thick sample for the Zr-Ti and the O atom columns, whereas the SrO and PbO atomic columns are relatively weak (20). The film-substrate interface was marked by depositing a layer of SrRuO_3 (SRO), nominally 1.5 unit cells thick, on STO prior to the deposition of PZT. The interface, denoted by a horizontal dashed line, is then determined by observing the plane of RuO_2 (which serves as a marker). A quantitative contrast analysis revealed that substitutional intermixing of Ru atoms occurred into the next upper (Zr-Ti) O_2 and into the next lower TiO_2 plane. For reasons of symmetry, the heterostructure was capped at the upper end by a SRO layer, nominally 1.5 unit cells thick, followed by a STO layer 15 unit cells thick.

The vertical shift in the Zr-Ti positions with respect to the adjacent O positions indicates that PZT is in a polarized state. On the left side of Fig. 1, this shift is upward, whereas on the right side it is downward, resulting in the polarization vector directions indicated by the arrows. This indicates the existence of two domains in a 180° orientation relation. By mapping the individual atom shifts, the position of the domain wall (dotted line) is localized. The horizontal width of this wall is about one $[\bar{1}\bar{1}0]$ projected crystal unit cell, which is in agreement with the results of earlier measurements (22). At the bottom interface, between the two 180° domains, in-plane displacements of the Zr-Ti positions with respect to the adjacent oxygen positions are observed. In-plane displacements have been predicted to play an important part in stabilizing such ferroelectric domain configurations (11).

Figure 2 displays an atomic displacement map superimposed on an image obtained by converting the grayscale contrast of Fig. 1 into a false-color representation. The arrows located at the Zr-Ti column positions indicate the modulus and the direction of the “off-center”

Fig. 1. Atomic-resolution image of a flux-closure structure with continuous dipole rotation in $\text{PbZr}_{0.2}\text{Ti}_{0.8}\text{O}_3$ (PZT) close to the interface to the SrTiO_3 (STO) substrate. The interface is marked by a horizontal dashed line (I), which is determined on the basis of a SrRuO_3 marker layer with a nominal thickness of 1.5 unit cells at the STO-PZT interface. The RuO_2 marker layer is also indicated. In the image recorded along the crystallographic $[\bar{1}\bar{1}0]$ direction, the atomic structure and the electric dipole direction (arrows) are given. In the image, two larger domains with 180° orientation can be identified. The domain wall is indicated by a yellow dotted line. In the center of the lower half of the image, an approximately triangular area (the domain wall is indicated by a dotted blue line) can be seen, where in the center the dipole direction makes an angle of 90° with the two large domains. The inset at the lower right shows a calculated image demonstrating the excellent match between the atomic model and the specimen structure.

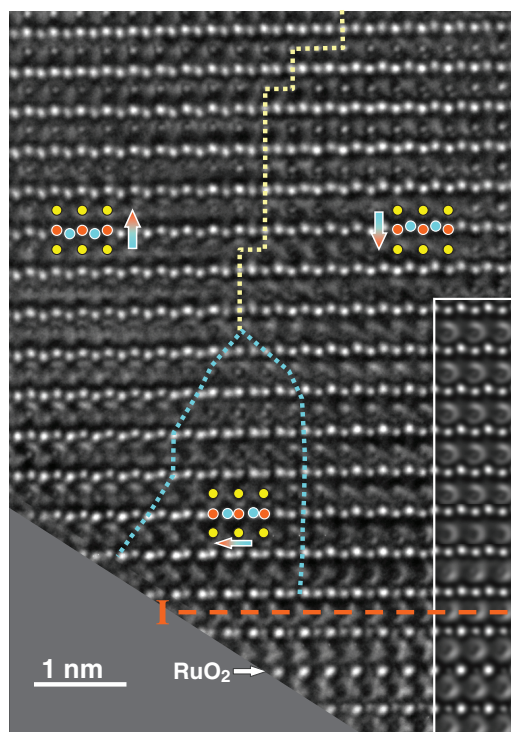


Fig. 2. Map of the atomic displacement vectors. The displacement of the Zr-Ti atoms (arrows) from the center of the projected oxygen octahedra is shown here superposed on the atomic image of Fig. 1. To enhance contrast, the gray scale is converted into a false-color representation. The length of the arrows represents the modulus of the displacements with respect to the yellow scale bar in the lower left corner. The arrowheads point into the displacement directions. Note the continuous rotation of the dipole directions from “down” (right) to “up” (left), which closes the electric flux of the two 180° domains.

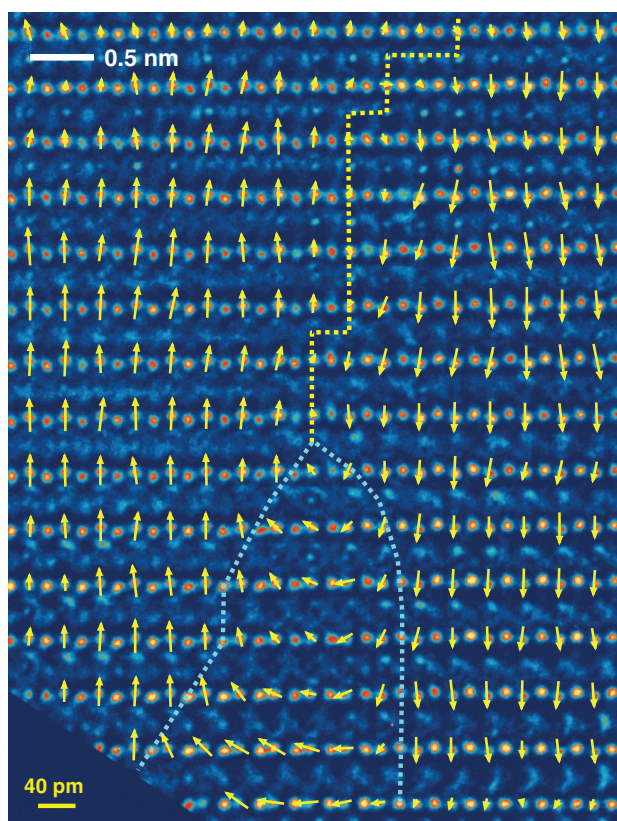
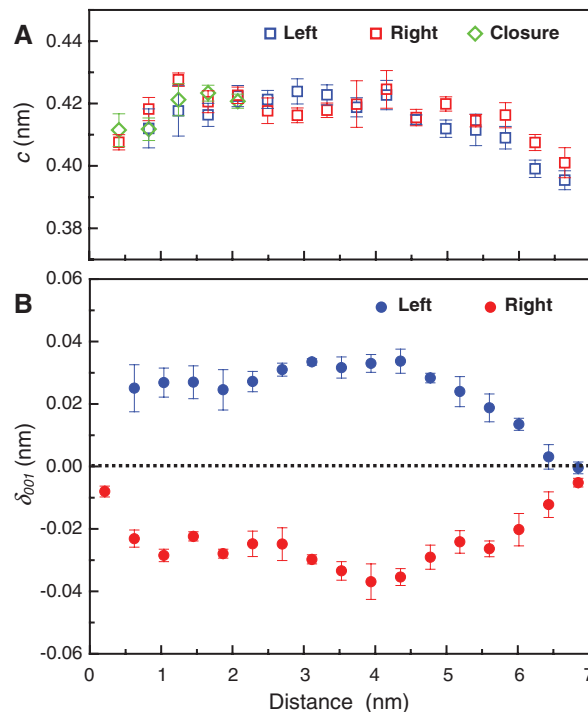


Fig. 3. (A) The vertical [001] lattice parameter c as a function of the distance from the lower interface. The value of c adopts a maximum value at the middle of the film layer and decreases when approaching the bottom and top interfaces. The behavior is the same in all three domain areas (red symbols for the left domain, blue for right domain, and green for flux-closure domain). (B) The shift parameter (δ_{001}), that is, the off-center displacements along the vertical [001] direction as a function of the distance from the lower interface. The blue symbols denote the upward displacements in the left domain; the red symbols indicate the downward displacements in the right domain. At the lower interface, the values change from the bulk value to a value close to zero within the distance of a single unit cell, whereas at the upper interface the shift parameter starts to decrease six unit cells away from the interface, reaching a value of zero there.



displacement with respect to the middle point of the horizontal line connecting the two neighboring O atom positions (taking the cubic structure with an inscribed oxygen octahedron in the nonpolar state as a reference). The scale at the lower left indicates a displacement of 40 pm. We note that a uniform atomic shift of this magnitude corresponds to an integral polarization of 108 $\mu\text{C}/\text{cm}^2$ (22, 26).

The displacement map provides direct evidence of a continuous rotation of the dipole direction from downward in the right-hand domain through a 90° orientation to upward in the left-hand domain, forming a particular type of flux-closure structure. The reorientation of the dipoles occurs within a well-defined area of triangular shape, with a maximum width of ~ 2.5 nm at the interface. The displacement vector modulus is small at the top, increasing toward the interface. The transition region from the downward orientation of the electric dipoles to the 90° orientation is about two projected unit cell widths on the right-hand side and up to about twice as wide on the left-hand side. No direct information is available on possible atom shifts along the $[\bar{1}\bar{1}0]$ viewing direction. However, the fact that we do not observe any in-plane elongation of the projected atom column contrast can be interpreted as evidence of the dipole rotation occurring exclusively in the image plane.

In Fig. 3, the [001] vertical crystal lattice parameter c and the modulus of the vertical displacements of the Zr-Ti atoms δ_{001} are displayed as a function of the distance from the STO-PZT interface to the interface at the capping STO layer (not shown in Figs. 1 and 2). The values were obtained by averaging, at a given vertical dis-

tance from the interface, the measured c values for the individual unit cells along the horizontal direction parallel to the interface. Starting (Fig. 3A) with a value of ~ 0.410 nm at the lower interface, c reaches a plateau value of 0.420 nm in the central part of the film layer before subsequently decreasing to 0.395 nm at the upper interface. The plateau and the upper-interface values are in good agreement with the values measured before for the fully polarized state and the depolarized state of PZT, respectively (22). This allows us to conclude that the presence of the flux-closure area at the lower STO-PZT interface reduces the depolarization field to such an extent that the polarization can essentially maintain its “bulk” value. On the other hand, the depolarization field at the upper interface (where we do not observe a flux-closure structure within the field of view) is so strong that polarization decays to a value close to zero. This conclusion is corroborated by the behavior of the shift parameter δ_{001} displayed in Fig. 3B. At the lower interface, both the left (upward-polarized) and right (downward-polarized) domains show a stable value on the order of 0.03 nm, typical for the polar state (22), down to the second horizontal atom plane (counted from the SRO marker plane). It is only in the first layer that the polarization decays. This behavior is in contrast to that in the direction to the upper interface, where δ_{001} starts to decrease six unit cells away from the interface, ultimately reaching a value of zero.

In bulk PZT, the c -axis lattice parameter and the atomic displacement of Zr-Ti and O show an equivalent behavior during the paraelectric-to-ferroelectric transition; that is, the value of both parameters increases. By contrast, we note that

inside the flux-closure area, c shows the same behavior as in the adjacent domains. This means that its value is essentially unaffected by the rotation of the electric dipoles of up to 90° , which in principle should lead to a reduction of the c -axis parameter and to an expansion perpendicular to this axis (i.e., parallel to the STO-PZT interface). This behavior can be understood as a consequence of the mechanical coupling to the neighboring 180° domains, whereupon the epitaxy constraints, because of lattice parameter misfit (27, 28), lead to a compressive stress in the PZT close to the interface.

Our results provide direct experimental evidence for the continuous rotation of the atomic-scale polarization dipoles over a distance directly relevant for the formation of vortex structures as predicted by theory (14, 15). Our observations agree well with the results of calculations for thin-film epitaxial systems by first-principles techniques, where the lateral extension of the flux-closure area was found to be quite narrow (8, 10, 29, 30), consistent with our own results. Furthermore, theory yields a continuous rotation of the polarization direction rather than a closure domain with a 90° polarization orientation and relatively narrow domain boundaries, as known for ferromagnetics.

The quantitative structural details of the flux-closure domain structure can be discussed in accordance with a thermodynamic theory (31) based on a Landau-Ginzburg-Devonshire free energy density taking into account polarization, mechanical stress, electric field, and domain wall structure. The equilibrium state, subject to consistency with Maxwell’s equations and linear elasticity theory, was determined for a ferroelectric PbTiO_3 heterostructure comprising a substrate and capping layer of (nonconducting) STO. The result was an approximately triangular flux-closure area with a basis in the interface on the order of a few nanometers. In addition, the depolarization field was quite strong at the top interface, essentially destroying polarization in the sample, although it was rather low in the environment of the flux-closure structure. This is confirmed by our study carried out at genuine atomic resolution, permitting access to the individual elementary electric dipoles.

With respect to potential charge screening at the SRO-containing interfaces, the following is essential. For SRO, a transition from metallic-conducting to insulating has been observed at layer thicknesses below four unit cells (32–34) and two unit cells (35). In the present case, the nominal SRO layer thickness at the PZT-STO interface is lower than these minimum thicknesses. In addition there is evidence of chemical intermixing destroying the chemical order, which is considered important for conductivity in these reference materials. Therefore, it can be concluded that the SRO layer used as a marker for the interface position is nonmetallic and thus cannot screen the depolarization field at the interface.

Continuous rotations of electric dipoles within flux-closure regions of ferroelectrics of the

type observed in this work were concordantly predicted by numerous theoretical treatments. The result (19) on vortex-like domain structures in rhombohedral materials such as BiFeO₃ corroborates our observations in the epitaxial PZT-STO system. We would like to point out that the vortex-like domain structures brought about by the comparatively simple 180°-90°-180° domain arrangement of our work are observed in films of highly tetragonal PbZr_{0.2}Ti_{0.8}O₃, where polarization rotation might be considered less likely than in rhombohedral materials like BiFeO₃. Both studies show that in perovskite-oxide heterostructures, polarization-closure structures do occur. Our work demonstrates the unique capabilities of mapping of the dipole vector, unit cell by unit cell, by means of atomic-resolution TEM. This technique can therefore be used to study further details of domain structures close to interfaces in ferroelectric heterostructures and of vortex-like structures in ferroelectric materials. Because these structures are sensitive to the balance between competitive interactions, even small changes in the interface properties could have substantial effects on the depolarization field behavior. In fact, theory predicts striking effects for thicknesses on the order of a few nanometers in the PZT layer, where the top and bottom layers (metallic or insulating) cannot be treated

separately (10). In addition, “dead” layers one unit cell thick (e.g., due to local deviations in permittivity caused by nonstoichiometry) are expected to change the flux-closure domain structure. Such investigations are also of potential importance for further progress in technology, in which increasingly thinner and smaller ferroelectric heterostructure systems are being considered for microelectronic applications.

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Vibrationally Quantum-State-Specific Reaction Dynamics of H Atom Abstraction by CN Radical in Solution

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Solvent collisions can often mask initial disposition of energy to the products of solution-phase chemical reactions. Here, we show with transient infrared absorption spectra obtained with picosecond time resolution that the nascent HCN products of reaction of CN radicals with cyclohexane in chlorinated organic solvents exhibit preferential excitation of one quantum of the C-H stretching mode and up to two quanta of the bending mode. On time scales of approximately 100 to 300 picoseconds, the HCN products undergo relaxation to the vibrational ground state by coupling to the solvent bath. Comparison with reactions of CN radicals with alkanes in the gas phase, known to produce HCN with greater C-H stretch and bending mode excitation (up to two and approximately six quanta, respectively), indicates partial damping of the nascent product vibrational motion by the solvent. The transient infrared spectra therefore probe solvent-induced modifications to the reaction free energy surface and chemical dynamics.

In a chemical reaction, the partitioning of energy between translational, rotational, vibrational, and electronic degrees of freedom of the products depends on, and therefore provides information about, the potential energy landscape over

which bonding changes occur (1). Early insights came from Polanyi (2), who demonstrated the importance of the location of an energy barrier along a reaction pathway in determining the fraction of the available energy that is released as product vibrational excitation. An expanding array of experimental techniques, complemented by theory, is enabling study of the dynamics of reactions in ever-increasing detail under low-pressure, gas-phase conditions in which the molecules are largely isolated from collisions and from the perturbations of a surrounding medium such as a solvent (1, 3–5).

Much synthetic, environmental, and biological chemistry occurs in solution, however, and the solvent will have a pronounced effect on the dynamics of chemical reactions (6–8). The very short time intervals between collisions in the liquid phase, and the hindered motions of molecules surrounded by a solvent cage, prevent application of many of the velocity- and quantum-state-specific experimental methods developed to examine gas-phase collisions (9). Spectroscopic methods using ultrafast lasers can be used to measure the time scales for reactions in solution (10–13), study solvent-solute complexes (14, 15), and examine molecular vibrational excitation, which can persist in solution for tens or hundreds of picoseconds (16). For a solution-phase bimolecular reaction, observation of vibrational quantum-state-specific energy disposal might provide comparable mechanistic insight to the infrared (IR) chemiluminescence (1, 2) and more recent velocity-map imaging (3) studies of gas-phase reactions and therefore unravel the influence of the solvent on the dynamics. This prospect was recognized by Hochstrasser and co-workers (10, 11), who used transient IR absorption to examine the products of reactions of Cl atoms or CN radicals with organic solvents. These pioneering experiments provided evidence that ~20% of the DCN products of the CN reaction with CDCl₃ solvent are formed with one quantum of vibrational excitation in the C-D stretching mode.

Here, experimental outcomes are presented for a solution-phase bimolecular reaction, which demonstrate a much greater degree of product vibra-

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tional excitation than was reported in prior studies of related reactions (10, 14, 15). As a consequence, vibrational mode-specific dynamics can be explored and are shown to be affected, but not quenched, by the presence of a solvent. The reaction of CN radicals with cyclohexane (Eq. 1),



and its deuterated counterpart are sufficiently exothermic that several vibrationally excited levels of the HCN (DCN) products are energetically accessible. Indeed, experimental studies of the gas-phase reactions of CN with small alkanes demonstrate efficient channeling of energy into as many as 6

quanta of the bending and 2 quanta of the C-H stretching vibrational modes of the HCN products (17–21). The three vibrational modes of HCN are well-described as a C-N stretch (ν_1), a bend (ν_2), and a C-H stretch (ν_3) (22), and are all IR active. These reactions therefore offer scope to examine how a liquid solvent alters the chemical dynamics by contrasting the nature and extent of vibrational excitation of the products in solution with the outcomes of gas-phase reactive collisions.

We obtained transient IR spectra with picosecond time resolution using the ULTRA laser facility at the Rutherford Appleton Laboratory (23). Figure 1 shows IR absorption spectra recorded at various time delays (≤ 400 ps) after narrow-

bandwidth IR excitation of the C-H or C-N stretching vibration of HCN in solution in CH_2Cl_2 . The spectra were obtained with broadband ($\sim 500 \text{ cm}^{-1}$) IR probe pulses and are a necessary precursor to the chemical reaction studies described later because they identify unambiguously the locations of bands of vibrationally excited molecules and their relaxation rates in solution. The features centered at 3263 and 3160 cm^{-1} are assigned, respectively, to the $\nu = 0 \rightarrow \nu_3 = 1$ and $\nu_3 = 1 \rightarrow \nu_3 = 2$ bands of HCN, hereafter denoted by 3_0^1 and 3_1^2 , respectively (24), and the features at 2094 and 2073 cm^{-1} are the 1_0^1 and 1_1^1 bands, respectively. The negative-going signals for the 3_0^1 band correspond to a bleach in the vibrational ground-state population that recovers through vibrational relaxation, and the positive-going signals for the 3_1^2 band derive from a transient population of $\nu_3 = 1$ that relaxes back to the ground state (25). Similar descriptions apply to the spectra of the 1_0^1 and 1_1^1 bands. Fits of the time-dependence of the integrated intensities of the 3_1^2 bands to bi-exponential functions yield time constants for the relaxation of $\text{HCN}(\nu_3 = 1)$ and recovery of $\text{HCN}(\nu = 0)$ in CHCl_3 , CH_2Cl_2 , and CDCl_3 of 130 ± 5 , 144 ± 8 , and 265 ± 20 ps, respectively. Corresponding analysis of the 1_1^1 band gives time constants for relaxation of the C-N stretch in these same three solvents of 157 ± 38 , 146 ± 17 , and 122 ± 20 ps. All uncertainties are 1 SD from fits to 3 to 6 data sets.

The results of reactive experiments for CN radicals, initiated by 266-nm, ~ 50 -fs ultraviolet (UV) laser photolysis of ICN (0.14 M) in the presence of cyclohexane (1.0 M) in CH_2Cl_2 solvent are shown in Fig. 2. The ultrafast dynamics of ICN photolysis in solution have previously been well characterized (26, 27). The spectra in the C-H stretching region show formation of both vibrationally excited and ground-state HCN products. The majority of the signal derives from reaction (1), with a weak contribution from reaction of CN radicals with the solvent, as shown in Fig. 2, bottom. The main bands occur at the same wavenumbers as those in Fig. 1A and are assigned as the 3_1^2 and 3_0^1 transitions of HCN,

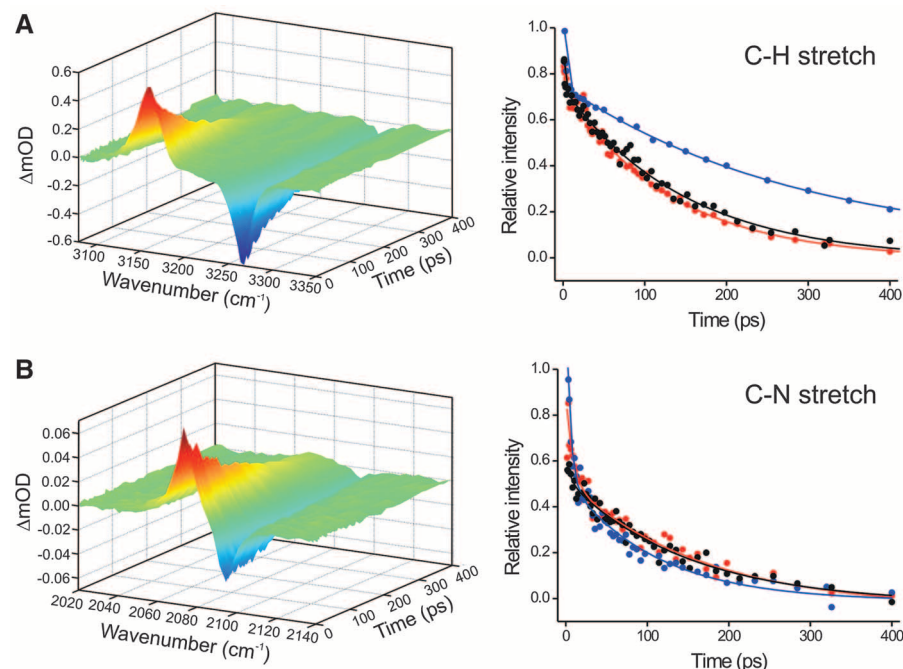


Fig. 1. Transient IR spectra of HCN in solution in CH_2Cl_2 (left) showing the temporal behavior in the (A) C-H and (B) C-N stretching regions after IR excitation on the fundamental absorptions (25). The right-hand plots show the time-dependence of the integrated intensities of the hot-band spectral features in three solvents, CH_2Cl_2 (black), CHCl_3 (red), and CDCl_3 (blue), with bi-exponential fits to extract time constants; the fast and slow decay components correspond respectively to rotational diffusion and vibrational relaxation.

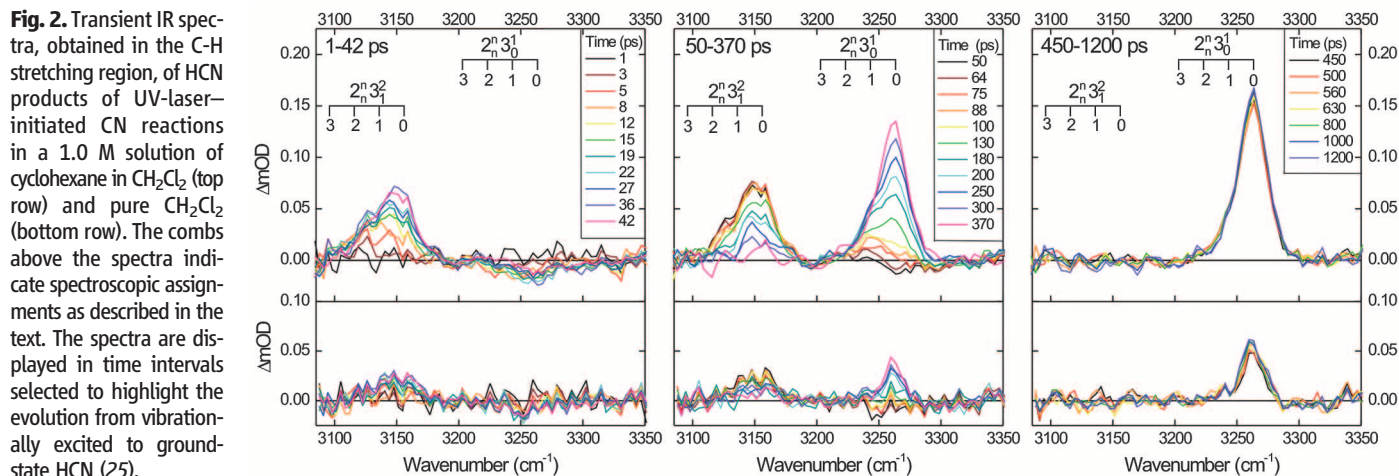


Fig. 2. Transient IR spectra, obtained in the C-H stretching region, of HCN products of UV-laser-initiated CN reactions in a 1.0 M solution of cyclohexane in CH_2Cl_2 (top row) and pure CH_2Cl_2 (bottom row). The combs above the spectra indicate spectroscopic assignments as described in the text. The spectra are displayed in time intervals selected to highlight the evolution from vibrationally excited to ground-state HCN (25).

but additional features appear to the low wave-number side of the main bands. These shoulders are assigned to diagonal hot bands in the HCN-bending vibration (2_n^n , with $n \geq 1$ denoting the number of quanta of excitation of the bend) observed in combination with the C-H stretching transitions. These $2_n^n 3_1^1$ and $2_n^n 3_0^1$ combination bands are slightly displaced from the 3_1^1 and 3_0^1 features by anharmonic shifting of the C-H stretching frequency and are observed because the reaction deposits energy in the HCN bending vibrational mode. The 3_1^1 and $2_n^n 3_1^1$ bands rise in intensity at earlier times than do the 3_0^1 and $2_n^n 3_0^1$ bands, but the 3_0^1 band becomes the sole spectral feature at longer time delays (25). Reaction (1) was also studied in CDCl_3 , which will exhibit different couplings to the reaction path and product motions, and similar HCN vibrational dynamics were observed (28). These observations are clear signatures that the reaction dynamics preferentially form HCN vibrationally excited with one quantum of C-H stretching motion and up to

two quanta of bending excitation, followed by vibrational relaxation to the ground state by coupling to the solvent bath. Weakly negative signals on the 3_0^1 band at early times are a consequence of a population inversion between $v_3 = 1$ and $v = 0$ vibrational levels. There is no evidence for the formation of HCN in higher vibrational levels of the C-H stretching and bending modes: The combs in Fig. 2 indicate where absorption features involving $v_2 = 3$ are expected, but these and the 3_2^2 band were not observed. Signal-to-noise ratios suggest an upper limit of 10% branching into $v_2 \geq 3$ and $v_3 \geq 2$ products.

The spectra for different pump-probe time delays were fitted to six Gaussian functions with centers fixed to the central wavenumbers of the $2_n^n 3_1^1$, 3_1^1 , $2_n^n 3_0^1$, and 3_0^1 bands ($n = 1$ and 2) and widths constrained to that of the 3_0^1 band at large time delays. For reaction (1) in CH_2Cl_2 , Fig. 3 displays the time-dependence of the resultant integrated peak areas. The $2_n^n 3_1^1$ intensity data have been combined, as have the intensities of

the $2_n^n 3_0^1$ bands, and the weak contribution from reaction with the solvent has been subtracted.

The time-dependent data were fitted to a kinetic model that incorporated reaction to form nascent $\text{HCN}(0n1)$, $\text{HCN}(001)$, $\text{HCN}(0n0)$, and $\text{HCN}(000)$ with respective pseudo-first-order rate coefficients (29) k_1 , k_2 , k_3 , and k_4 . The terms in parentheses denote numbers of quanta of excitation of the modes ($v_1 v_2 v_3$). The model also included vibrational relaxation in steps of a single quantum (with rate coefficients k_4 to k_8). It has analytical solutions for the time dependence of the concentrations of $\text{HCN}(0v_2 v_3)$ to which the data were simultaneously fit. Allowance was made for the dependence of IR absorption signals on the population difference between levels connected by a spectroscopic transition, and the fits incorporated differences in the transition dipole moments for HCN with zero and one quanta of C-H stretch (30). The fits were further constrained by fixing the rate coefficients for loss of a quantum of C-H stretch to the values derived from the IR-pump-and-probe experiments for HCN in CH_2Cl_2 . Fitted values of k_1 through k_4 are displayed in Fig. 3 and can be interpreted as being proportional to the reactive branching to form nascent $\text{HCN}(0n1)$, $\text{HCN}(001)$, $\text{HCN}(0n0)$, and $\text{HCN}(000)$ products. The values indicate that the reaction preferentially forms HCN with a quantum of C-H stretch and additional bending excitation ($v_2 \leq 2$); the main source of population of $\text{HCN}(000)$ is through vibrational relaxation by coupling to the solvent over time scales of ~ 130 to 270 ps.

Reactions of CN with alkanes release $\sim 10000 \text{ cm}^{-1}$ of energy, and experimental investigations of such reactions in the gas phase (17–21) demonstrated that the excess energy is efficiently coupled into certain internal motions of the products: There is substantial excitation of both the HCN bending mode (up to $v_2 \sim 6$) and the C-H stretching mode ($v_3 \leq 2$). Our quasi-classical trajectory calculations for isolated reactive collisions indicate that the bending excitation of the HCN stems from a flat bending potential in the vicinity of the transition state (TS) (31), although the high rotational excitation of CN radicals from UV photo-

Fig. 3. (Top) The time-dependence of the integrated intensities of spectral bands in the C-H stretching region of HCN formed from reaction (1) in solution in CH_2Cl_2 : Blue is $\text{HCN}(0n1)$ ($n = 1, 2$), red is $\text{HCN}(001)$, purple is $\text{HCN}(0n0)$, and black is $\text{HCN}(000)$. Solid lines show fits to the (Bottom) summarized kinetic model, which also displays values of the rate coefficients obtained for each step. Error bars on individual data points are ± 2 SD from the least-squares fitting to band intensities; uncertainties in rate coefficients are 1 SD from fits to four data sets.

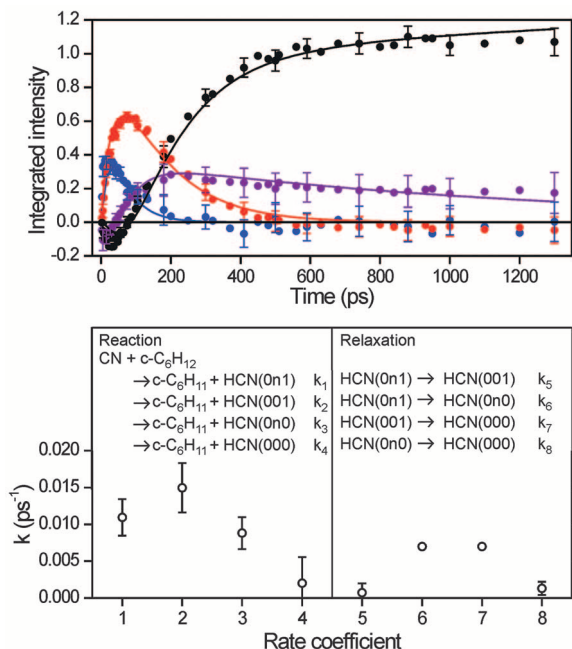
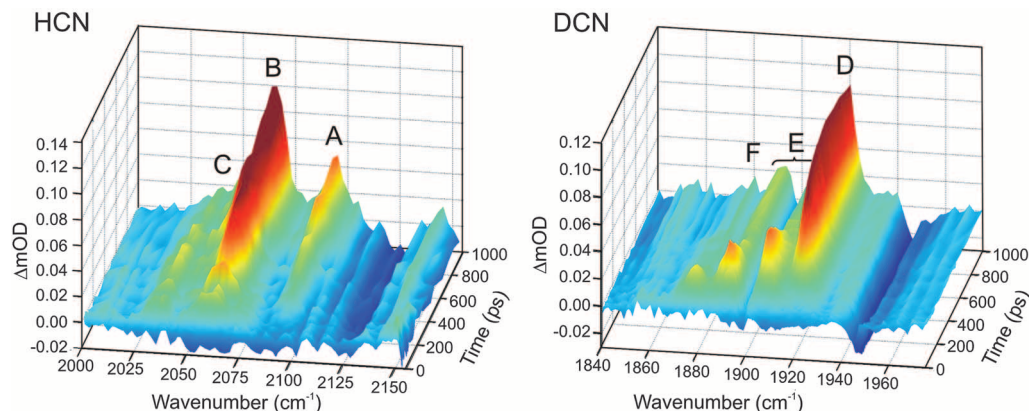


Fig. 4. Time-dependent IR spectra obtained in the C-N stretching regions of HCN and DCN for UV-laser-initiated CN reactions with C_6H_{12} in CH_2Cl_2 and C_6D_{12} in CHCl_3 . Features (A) and (D) are the 1_0^1 bands in HCN and DCN, respectively. Bands (B) and (C), centered at 2065 and 2037 cm^{-1} , are assigned, respectively, to INC and to CN radicals, which may be complexed with the solvent. For HCN, hot bands of v_1 and combinations of the 1_0^1 transition with hot bands of the other two modes overlap band (B); for DCN, these bands are in the region indicated as (E). Band (F) is discussed in the text. The dips in the DCN spectrum at 1899 [at the center of region (E)] and 1946 cm^{-1} are solvent absorption-induced transient signals (25).



dissociation of ICN (26) may contribute. Early H-atom transfer at an extended C-H distance causes the observed excitation in the C-H stretching mode. The condensed phase experiments observe lower vibrational excitation of HCN than was reported for isolated collisions in the gas phase, however. We deduce that the solvent partially suppresses the flow of the excess energy of the reaction into product vibration, but that despite the presence of the solvent, many features of the dynamics on an attractive potential energy surface with an early barrier and loose bending potential persist. Calculations indicate that the location of the TS is not much affected by solvation, and the reduction in the vibrational content of the HCN therefore derives from solvent friction in the post-TS region (31).

Figure 4 shows transient IR spectra in the C-N stretching region of HCN and DCN products from CN radical reaction with $c\text{-C}_6\text{H}_{12}$ in CH_2Cl_2 and $c\text{-C}_6\text{D}_{12}$ in CHCl_3 , respectively. Spectral features are assigned to the 1_0^1 C-N fundamental band and, at early times, to bands involving this transition in combination with hot bands in the bending and C-H (or C-D) stretching vibrations (in accord with the aforementioned promotion of excitation in these modes by the chemical dynamics). For the CN + $c\text{-C}_6\text{H}_{12}$ reaction, there is no firm evidence of excitation of the C-N stretch in the HCN product, but analysis is complicated by features at 2065 and 2037 cm^{-1} assigned, respectively, to INC (32) and CN [either as free radicals in solution or weakly complexed with solvent molecules (14, 15)]. The C-N stretching region of DCN is free from the above interferences, and an additional, weak transient feature (Fig. 4F) is seen at 1877 cm^{-1} . A plausible assignment to the $1_1^2 3_1^1$ or $1_1^2 2_2^2 3_1^1$ combinations of hot bands is consistent with the reaction dynamics channeling energy into the C-D stretching and bending motions but also indicates some C-N stretching activity in reactions forming DCN. This latter observation may be a consequence of subtly different reaction dynamics for the D-atom abstraction or more mixed C-D and C-N stretching character of the normal vibrational modes of DCN.

In conclusion, transient IR absorption spectroscopy has shown that the dynamics of the reactions of CN radicals with cyclohexane in chlorinated solvents have many features in common with their gas-phase counterparts. The HCN products are formed with high degrees of vibrational excitation (on average, ~30% of the available energy), and ground-state HCN mostly results from vibrational relaxation via coupling to the solvent on a slower time scale. The dynamics are vibrationally quantum-state-specific, with preferential excitation of a single quantum of the C-H stretching mode and up to two quanta in the bending mode. This degree of vibrational excitation is, however, lower than has been reported for comparable reactions in the gas phase, indicating partial damping of the developing HCN vibrational motion after the transition state. The transient IR spectra illustrate a strategy to explore not only the ways

in which a solvent modifies the potential energy landscape for a chemical reaction in solution but also constrains the chemical reaction dynamics.

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Regeneration of Ammonia Borane Spent Fuel by Direct Reaction with Hydrazine and Liquid Ammonia

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Ammonia borane ($\text{H}_3\text{N-BH}_3$, AB) is a lightweight material containing a high density of hydrogen (H_2) that can be readily liberated for use in fuel cell-powered applications. However, in the absence of a straightforward, efficient method for regenerating AB from dehydrogenated polymeric spent fuel, its full potential as a viable H_2 storage material will not be realized. We demonstrate that the spent fuel type derived from the removal of greater than two equivalents of H_2 per molecule of AB (i.e., polyborazylene, PB) can be converted back to AB nearly quantitatively by 24-hour treatment with hydrazine (N_2H_4) in liquid ammonia (NH_3) at 40°C in a sealed pressure vessel.

A critical factor in realizing a hydrogen (H_2) economy is storage of the molecule for controlled delivery, presumably to an

energy-producing fuel cell (1). Options for H_2 storage include compressed hydrogen, metal hydrides (2), and porous sorbent materials (3). In addition

to these, there is also the possibility of using chemical hydrides wherein the H_2 is stored in E–H bonds (E: C, B, N, or O). In this case, hydrogen can be released thermally or through the use of an acid, base, or metal catalyst. Ammonia borane (H_3N-BH_3 , AB) is a particularly appealing chemical hydrogen storage medium, owing to its high gravimetric capacity of H_2 (19.6 weight percent), low molecular mass (30.7 g mol^{-1}), and overall chemical properties (1). AB has both hydridic and protic hydrogens, facilitating H_2 release under mild conditions. Methods explored for promoting release include acid or base treatment, as well as transition-metal catalysis (4–8). At the same time, the viability of any storage system is critically dependent on efficient recyclability, but reports on the latter subject are sparse and the processes generally energy intensive (1, 9–13).

The composition of spent fuel depends on the dehydrogenation method used (1, 14–16). Initial transition metal-catalyzed dehydrogenation of AB with rhodium catalysts such as $[Rh(cod)Cl]_2$ (cod: 1,5-cyclooctadiene) produced the intermediates cyclotriborazine and μ -aminodiborane before forming borazine, polyborazylene (PB, Fig. 1), and other oligomers as the final dehydrogenated material (17, 18). More recent transition-metal catalyst systems based on $Ir(H_2)POCOP$ [POCOP: κ^3 -1,3-(OP^tBu)₂C₆H₃] form oligomeric polyaminoboranes (19) as the exclusive product, but these are limited by the release of only one equivalent of H_2 (5). However, transition metals supported by *N*-heterocyclic carbenes (NHC), e.g., $Ni(NHC)_2$ complexes, can release greater than two equivalents of H_2 and give PB as a single spent-fuel component (6). We have predominantly pursued methods for regenerating PB as this is the form of spent fuel that results from loss of greater than two equivalents of H_2 per molecule of AB. Nonetheless, the presence of residual catalyst in PB mixtures could complicate the AB regeneration process, so we have used a purer form of PB that is derived from dehydrogenation of borazine. PB is not a simple polymeric material, instead consisting of a mixture of oligomers. Consequently, PB can exhibit variation in molecular weight distribution and empirical formulae depending on the conditions used for its synthesis, and is composed of partially cross-linked structures and structures containing fused or polycyclic ring systems. As a result of these factors, the ^{11}B NMR (nuclear magnetic resonance) spectrum of PB exhibits a broad, featureless resonance centered around 31 parts per million in tetrahydrofuran (THF) due to the range of boron environments present (20, 21).

An important consideration here is that both catalytic and thermal (i.e., noncatalytic) routes to PB from AB (based on the volume of H_2 evolved) yield a hydrogen-depleted material in which greater than two equivalents of hydrogen are released from AB. The PB generated in either methodology to this point appears to be indistinguishable from the other. This leads to the general acceptance of the formulation of PB as “ BNH_x ” where x is greater than zero, less than two, but typically one (20). This material thus corresponds to a spent fuel derived from the loss of greater than two equivalents of H_2 per molecule of AB, but still allows for the preservation of some of the energetic B–H bonds. This latter property confers both solubility for subsequent recycle chemistry as well as reduction of the overall energy required to regenerate the desired BH_3 fragments. Although synthetically prepared PB derived from borazine is a surrogate for real spent fuel, it represents a scenario in which as much H_2 has been liberated downstream of AB before ceramic BN is formed. This makes it an ideal material with which to demonstrate the conversion of this spent fuel form to AB.

Owing to the thermodynamics of AB dehydrogenation, in which the energy required to dehydrogenate AB to PB, liberating 2.5 equivalents of hydrogen, is $-57.7 \text{ kJ mol}^{-1}$ (22–25), direct hydrogenation of spent fuel would require a substantial energy input (4, 22–25). Because a simple on-board direct regeneration is unlikely, a more viable approach would be to perform the regeneration off-board in a series of reactions that are more thermodynamically reasonable. We recently described (13) the use of *ortho*-benzenedithiol (BDT) for recycling PB. This approach avoided the formation of thermodynamically stable B–O bonds, moieties that require high-energy reducing agents to reform the BH_3 units (recycling B–O bonds is somewhat analogous to recycling CO_2 to gasoline). This sulfur-based reagent was selected because B–S bonds are weaker than the analogous B–O bonds, and the acidity of the S–H moiety was likely to facilitate breakdown of the polymeric PB structure into molecular fragments for subsequent reduction chemistry. The process proved successful on a 50-mg scale with subsequent conversion of the (BDT)B–H species back into AB, which was accomplished with Sn–H reagents (26). To understand how this approach might translate from the

laboratory to an industrial plant environment (100 metric tons/day recycle of AB), an economic analysis was carried out on the thiolate/tin based chemistries (27). This assessment showed that the mass of the tin reductants (e.g., Bu_3SnH , $291.06 \text{ g mol}^{-1}$) was a major contributor to the overall plant cost due to the physical transport of this high molecular weight material. It was therefore apparent from this analysis that one way to increase the process efficiency would be to use a substantially lighter reductant.

On the basis of this information, we thus endeavored to find a simplified, more energy-efficient process for AB recycle involving a minimum number of steps. In considering potential reductants for this purpose, hydrazine (N_2H_4 , 32.05 g mol^{-1}) appeared to be a good candidate because of its low molecular weight. In the sense of a redox reaction, hydrazine can decompose as $N_2H_4 \rightarrow N_2 + 2H^+ + 2H^-$ resulting in two protons and hydridic hydrogen atoms and diatomic nitrogen gas (N_2) as the likely potential by-product of any reduction chemistry, which lends itself well to potential capture and recycle. Because PB is soluble in polar organic solvents, our initial studies using N_2H_4 were carried out in THF solution at room temperature (28). The PB was converted by the hydrazine to species that only contained BH_3 units. However, the major product was hydrazineborane ($N_2H_4-BH_3$, HzB), with smaller amounts of AB as determined by $^{11}B\{^1H\}$ NMR even when substoichiometric amounts of hydrazine were used and residual PB remained. The development of HzB as a potential H_2 storage material has been investigated. The first report on the thermal decomposition of HzB was by Ricker and Goubeau in 1961 (29), and more recently Lentz and co-workers improved the H_2 -release behavior by addition of LiH (30). However, one main problem highlighted in this later report is the inability to identify the products after dehydrogenation and the subsequent lack of regeneration strategies for this unknown material. It therefore became apparent that conversion of HzB into AB would be a key reaction to achieve appreciable regeneration of a H_2 storage material. When HzB was dissolved in a vast excess of liquid NH_3 at room temperature ($\sim 150 \text{ psi}$ at 25°C), no exchange of the N_2H_4 for NH_3 was observed; rather, only HzB was observed in the ^{11}B NMR spectrum, even after 14 days. Surprisingly, direct heating of

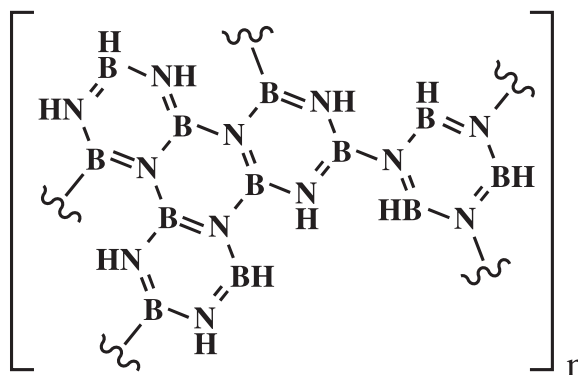


Fig. 1. A representative structure of PB.

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H₂B in a 0.5 M NH₃ solution in dioxane gave improved yields of AB, up to 25% as indicated by ¹¹B NMR spectroscopy. Thus, we conjectured that energy in the form of heat was necessary to aid the scission of the B–N bond in H₂B with subsequent formation of AB, and we attempted the reaction of H₂B in a sealed vessel with liquid NH₃ (60°C) for 24 hours. After the NH₃ was allowed to evaporate, a trace amount of N₂H₄ remained, and the predominant boron-containing species was AB (~85% yield by ¹¹B NMR spectroscopy), the only other observable boron-containing species being H₂B. If the reaction work-up conditions were changed to include removal of the volatiles (including remaining hydrazine, boiling point 114°C, vapor pressure 14.4 torr at 25°C), under high vacuum the yield could be improved to 95% isolated ammonia borane. The residual hydrazine does play a role in the conversion of AB to H₂B. The exchange in neat hydrazine shows complete conversion to H₂B after 12 hours at 60°C, and this reaction is slowed as the temperature is lowered (2% after 12 hours at 25°C) (28), consistent with some form of kinetic control. These results are consistent with high-level CCSD(T)-complete basis set calculations, which show that the B–N dative bond energy in AB is 4.5 kcal/mol less than the B–N dative bond energy in H₂B at 298 K. Using a self-consistent reaction field approach (31) with the COSMO (conductor-like screening model) parameterization (32) as implemented in two different electronic structure packages (Gaussian and ADF) (33–35), we calculated the energy of the reaction in NH₃, THF, and dioxane. The two computational implementations differ slightly, but in both cases the free energy for the exchange reaction is substantially decreased in NH₃ as a solvent with free-energy differences of 1.9 to 1.0 kcal/mol. As the dipole moment of the solvent decreases, the free energies approach the gas-phase value. Thus, BH₃NH₃ is more stabilized in the polar solvents, leading to a decrease in the free energy.

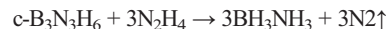
This result led us to hypothesize that, if in THF solution H₂B was the predominant product in the reaction of PB with N₂H₄, the coordinated N₂H₄ moiety should be readily displaced from any N₂H₄–BH₃ formed in situ with liquid NH₃ at elevated temperatures. This would drive the formation of the desired AB product while freeing N₂H₄ for further reduction of the B–H bonds in unreacted PB. This procedure would not only enable us to minimize the amount of N₂H₄ required to recycle AB, but also greatly improve production and recycle costs by reducing separation steps, the only by-product being N₂, which itself could potentially be recovered. Indeed, carrying out the reaction of PB and N₂H₄ in liquid NH₃ under slightly milder conditions (40°C) for 24 hours resulted in quantitative conversion to BH₃-containing species. ¹¹B NMR spectroscopic investigation of the crude material after removal of NH₃ and the remaining N₂H₄ under dynamic vacuum revealed quantitative conversion to BH₃

compounds, with ~92% being AB with the remainder as H₂B (Fig. 2). Differential scanning calorimetry on the AB produced by this method afforded identical results to that performed on an authentic sample of AB (36). In an ideal situation, 4 “BNH” + 5N₂H₄ → 4H₃N–BH₃ + 5N₂, meaning that every 1 mol of PB requires 1.25 mol of N₂H₄ to fully regenerate AB from spent fuel. This reaction energy can be estimated to be –64.7 kcal/mol for the production of solid AB from liquid “BNH” [see (28) for details]. As a result, a slight excess of hydrazine was used (1.35 equivalents of N₂H₄) to compensate for differing extents of cross-linking in PB. After AB regeneration, the NH₃/N₂H₄ that is removed from the reaction can be readily recycled and, if necessary, a straightforward separation of these two components can be carried out.

To confirm the necessity of hydrazine as a reductant, we also heated PB in liquid NH₃ at 60°C in the absence of N₂H₄ for 14 days. We observed no conversion to AB, and only starting material was recovered at the end of the reaction period.

To further understand the mechanism of this conversion, we calculated the energies of the intermediates for the addition of N₂H₄ molecules

to a reasonable model system [i.e., “BNH₂” (borazine)] at the G3MP2B3 level (37) of theory (28). The overall reaction sequence is complicated owing to the presence of many reaction steps (more than a hundred reactions). The computational results indicate that the initial reactions that lead to decomposition of the ring are near thermoneutral and that subsequent reactions are exothermic. The overall reaction for conversion of “BNH₂” is



and the reaction is slightly endothermic ($\Delta H = 5.7$ kcal/mol) in the gas phase and exothermic ($\Delta H = -52.5$ kcal/mol) if the *c*-B₃N₃H₆ and BH₃NH₃ are in the solid state (using the best available values).

For this route to become an industrially viable process for H₂ storage, the worldwide production volume of hydrazine would need to be either increased or produced via a new process. Hydrazine is produced primarily by the Olin-Raschig process (from NH₃, NaOH, and Cl₂) as an aqueous solution for less than \$4 a kilogram and has a wide variety of industrial uses, including in nuclear fuel reprocessing, as a fuel in rocket

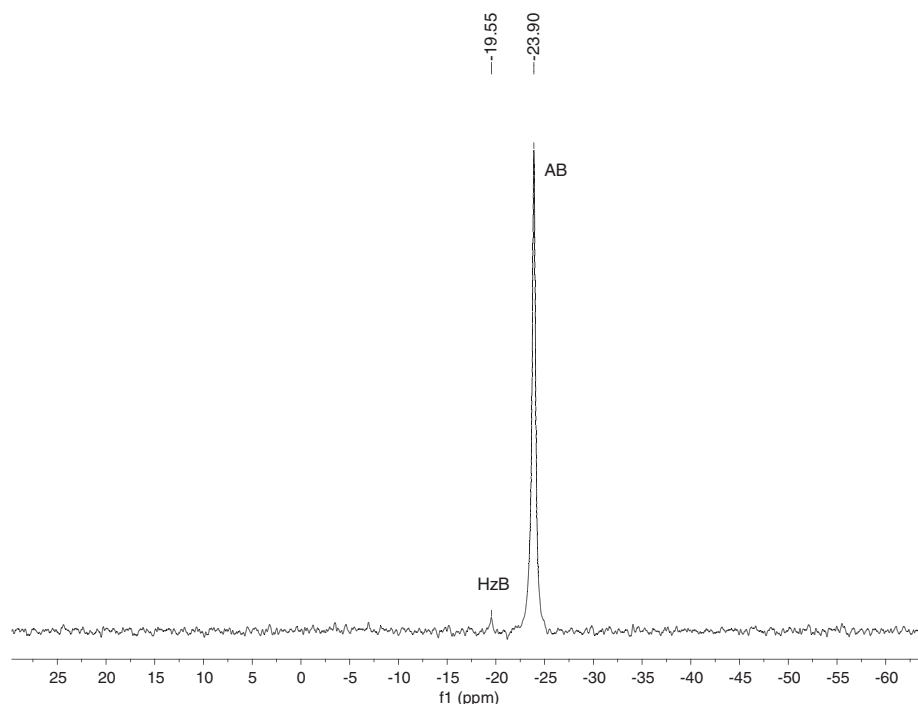
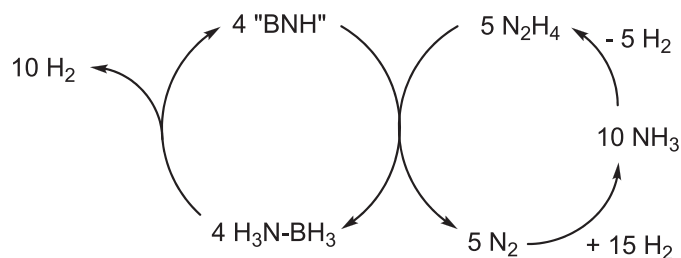


Fig. 2. ¹¹B{¹H} NMR spectrum in THF for crude material isolated from one-pot PB/N₂H₄/NH₃ reaction after heating at 40°C for 24 hours, resulting in H₂B (8%) and AB (92%).

Fig. 3. Ideal overall reaction scheme for AB (H₃N–BH₃) regeneration from PB (“BNH”) with hydrazine (N₂H₄).



propulsion, as a boiler feedwater deoxygenating agent, and in the manufacture of foamed plastics, pharmaceuticals, and biodegradable pesticides and herbicides (38). However, with worldwide production capacity estimated to be ~200,000 metric tons/year in 2007, the limited production volume and limited applications make current pricing less relevant. Hydrazine production colocated with recycle plants would reduce the transportation and storage costs and reduce the cost of hydrazine further, although a more efficient method for hydrazine synthesis would be of greater benefit.

The overall reaction cycle, including the production of hydrazine, is summarized in Fig. 3. In this scheme, the N_2 released from the N_2H_4 can be rehydrogenated to NH_3 and subsequently converted to N_2H_4 . Although synthetic procedures such as the Olin-Raschig process produce hydrazine in high yields and great efficiency, we can begin to look to the future and think about a new route to hydrazine production. In conjunction with an AB-regeneration plant, in situ N_2H_4 production from NH_3 does not require high conversion rates to produce a N_2H_4/NH_3 feed for AB regeneration. In this scheme, the only material consumed is hydrogen, the production of which is widely recognized as the second major technical challenge for the hydrogen economy.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/331/6023/1426/DC1
Materials and Methods
Figs. S1 to S5
Tables S1 to S4
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Dynamic Control of Chiral Space in a Catalytic Asymmetric Reaction Using a Molecular Motor

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Enzymes and synthetic chiral catalysts have found widespread application to produce single enantiomers, but in situ switching of the chiral preference of a catalytic system is very difficult to achieve. Here, we report on a light-driven molecular motor with integrated catalytic functions in which the stepwise change in configuration during a 360° unidirectional rotary cycle governs the catalyst performance both with respect to activity and absolute stereocontrol in an asymmetric transformation. During one full rotary cycle, catalysts are formed that provide either racemic (*R,S*) or preferentially the *R* or the *S* enantiomer of the chiral product of a conjugate addition reaction. This catalytic system demonstrates how different molecular tasks can be performed in a sequential manner, with the sequence controlled by the directionality of a rotary cycle.

The ease with which nature controls the chirality of the product of an enzymatic reaction has spurred and inspired the development of synthetic compounds that enantio-

selectively catalyze a myriad of asymmetric transformations (1, 2). The precise spatial arrangement of amino acid and cofactor subunits in a highly dynamic setting is the key to enantioselectivity

achieved by enzymes, yet the chirality of the active site environment is set by the inherent chirality of the constituent amino acid building blocks. As a result, it is very challenging to switch an enzyme's selectivity from favoring a product of one chiral sense to favoring its enantiomer, although genetic engineering and directed evolution approaches can be brought to bear in certain cases (3). By contrast, the comparative structural simplicity of synthetic asymmetric catalysts often allows facile preparation of both catalyst enantiomers, thereby enabling preparation of each product enantiomer individually (4). In addition, strategies that allow for the formation of either product enantiomer from a single enantiomer of a catalyst have also been developed, for example, by changing the reaction conditions, including solvent or temperature, or by the addition of different Lewis acids (5, 6). But can we go further; can we modulate

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the chiral sense of the space in which a catalyzed reaction takes place in a dynamic and responsive manner using an external stimulus?

Here, we show that an external signal, that is, light, can be used to control the chiral space in which a catalytic reaction takes place. In our design, a single enantiomer of a molecular catalytic system can be triggered in situ to produce a racemate, one enantiomer, or the other enantiomer of a chiral product on demand.

We demonstrate the basic principle of such a chiral catalytic system by exploiting the unique dynamic stereochemical features of unidirectional rotary molecular motors (7–9). During the individual steps of a rotary cycle of a light-driven molecular motor, the stereochemistry changes several times. This variability provides a scenario for the design of a motor with integrated catalytic functions in which the stereocontrol during the catalytic event can be alternated by light in a sequential manner. The individual chiral stages during the unidirectional rotary cycle dictate the ability of the catalyst to induce a preferred handedness in the product formed during an asymmetric transformation.

The design principle, in which motor and catalytic functions are integrated, is shown in Fig. 1 (top). A light-driven molecular motor is functionalized at the rotor and stator segments with moieties A and B, respectively, which can cooperate to form a bifunctional organocatalyst (10, 11) when in proximity to each other. The helicity of the motor unit, which dictates the overall chirality of the system, changes during the 360° rotary cycle. Simultaneously, the precise positioning of the two catalytic moieties A and B in space, both with respect to distance and helical organization, is governed by rotor and stator orientation at each stage of the rotary cycle. A and B can be remote (stage I), in close proximity with *M* helicity (stage II), or in close proximity with *P* helicity (stage III). As a consequence, the cooperative effect of A and B and the preferred chiral sense induced in a transformation catalyzed by the system is modulated by the rotary cycle.

In contrast to other light-gated systems (12), this adaptive chiral catalyst allows for exquisite control over both catalytic activity and stereoselectivity in a concerted and fully reversible manner.

The structure of the molecular motor-based catalyst is shown in Fig. 1 (bottom). The molecule is a single enantiomer in which *R* denotes the absolute configuration at the methyl-substituted indanyl centers, *P* the helicity of the indane pair, and *trans* the orientation of the two pendant functional groups (A and B in Fig. 1) about the central olefin. The design features a chiral overcrowded alkene that can perform a unidirectional four-stage rotation cycle fueled by light irradiation (7, 13, 14). The catalytic moieties are modeled after recently developed organocatalysts (15–17) and are connected to the motor via para-phenyl spacers at the stator and rotor units of the motor. They comprise a DMAP (dimethylaminopyridine) Brønsted base (A) and a thiourea hydrogen-bonding

donor group (B) to ensure cooperative action in organocatalytic Michael additions (16, 17). However, before addressing the issue of control of catalytic function, the proper operation of the unidirectional rotary motor has to be established.

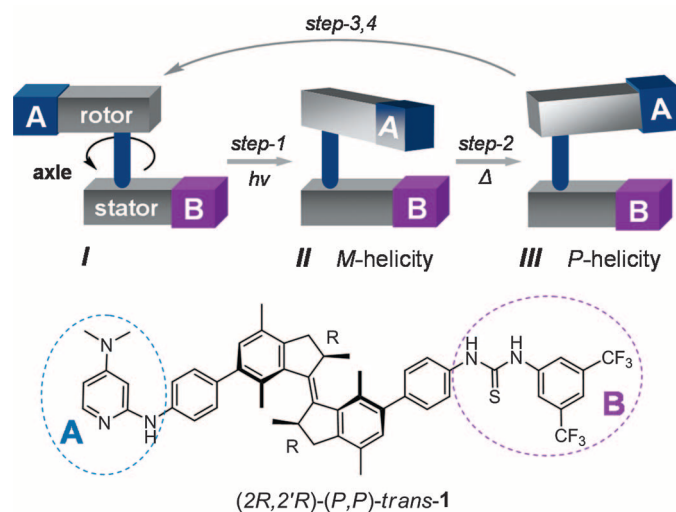
The syntheses of (2*R*,2'*R*)-(*P*,*P*)-*trans*-1 and its enantiomer (2*S*,2'*S*)-(*M*,*M*)-*trans*-1 are detailed in figs. S1 to S6 and notes S1 to S3. Unless indicated otherwise, the (2*R*,2'*R*)-(*P*,*P*) enantiomer was used to obtain the results that follow. Unidirectional clockwise rotation of the rotor with respect to the stator in motor 1 was confirmed by a combination of ultraviolet-visible (UV-vis), circular dichroism (CD), and proton nuclear magnetic resonance (¹H-NMR) spectroscopy and chiral high-performance liquid chromatography (HPLC). Upon exposure of enantiomerically pure (*P*,*P*)-*trans*-1, dissolved in tetrahydrofuran (THF), to UV irradiation ($\lambda^{\text{irr}} = 312 \text{ nm}$) at 20°C, the intensity of the absorption band at 300 nm decreases in concert with the emergence of a red-shifted absorption at 360 nm (Fig. 2A). The spectral change was assigned to a (*P*,*P*)-*trans*-1 $\rightarrow$ (*M*,*M*)-*cis*-1 interconversion (Fig. 2J) ($\Phi^{\text{iso}} = 0.37$) with a photo-stationary state comprising >99% of (*M*,*M*)-*cis*-1. The formation of (*M*,*M*)-*cis*-1 was also evident from chiral HPLC (see fig. S7) and ¹H-NMR analysis (Fig. 2I) and the observed CD spectral changes (Fig. 2E) typically associated with the *P*,*P* to *M*,*M* helix inversion (7). When the solution of (*M*,*M*)-*cis*-1 was subsequently heated at 70°C for 40 min, a thermal isomerization step (standard Gibbs energy of activation $\Delta^\ddagger G^\circ = 104.1 \text{ kJ/mol}$; for the kinetic data and analysis, see fig. S8) resulted in the formation of (*P*,*P*)-*cis*-1 (Fig. 2J), as indicated by the decrease in absorption at 360 nm (Fig. 2B). The concomitant helix inversion of the *cis* isomer of 1 from *M*,*M* to *P*,*P* is supported by ¹H-NMR (Fig. 2I), chiral HPLC (see fig. S7), and CD (Fig. 2F) measurements.

Quantitative analysis of these data revealed that 76% of (*P*,*P*)-*cis*-1 was obtained after this isomerization step. Two subsequent photochemical and thermal isomerization steps, 3 and 4 (Fig. 2J), of (*P*,*P*)-*cis*-1 resulted in the formation of the original (*P*,*P*)-*trans*-1 isomer, completing the four-stage cycle. In this final sequence, the photochemical (*P*,*P*)-*cis*-1 to (*M*,*M*)-*trans*-1 isomerization ($\lambda^{\text{irr}} = 312 \text{ nm}$, -60°C , $\Phi^{\text{iso}} = 0.18$, 81%) provides a highly unstable isomer that undergoes rapid (-10°C , 100%) unidirectional thermal isomerization to the initial (*P*,*P*)-*trans*-1 isomer. Again the structural changes and helix inversions during these isomerization processes could be followed by UV-vis (Fig. 2, C and D) and CD (Fig. 2, G and H) spectroscopy and chiral HPLC (see fig. S7), confirming that the initial (*P*,*P*)-*trans*-1 isomer was formed after the complete cycle.

The UV-vis spectral changes during these isomerizations show distinct isosbestic points (332 nm, step 1; 329 nm, step 2; 318 nm, step 3; 326 nm, step 4), indicating selective unimolecular processes. Each photoisomerization is accompanied by a red-shifted absorption consistent with the formation of a high-energy isomer due to enhanced strain in the helical structure. The subsequent energetically downhill thermal isomerization steps result in a more relaxed structure and govern the unidirectional nature of the entire rotary cycle.

Having established the four-step unidirectional rotary cycle of 1, we investigated its performance as a chiral organocatalyst, using the enantioselective Michael addition of 2-methoxy thiophenol 2 to cyclohexenone 3 as a model reaction (Fig. 3A) (17). Product formation was monitored by ¹H-NMR in CD₂Cl₂ at -15°C with a catalyst loading as low as 0.3 mole percent (mol %) (see note S4). The enantiomeric ratio (e.r.) and absolute configuration of product 4 were determined by chiral HPLC (see Fig. 3, C to E). When the (*P*,*P*)-*trans*-1 isomer

Fig. 1. Schematic illustration of an integrated unidirectional light-driven molecular motor and bifunctional organocatalyst (top) and the molecular structure of (2*R*,2'*R*)-(*P*,*P*)-*trans*-1 (bottom). The motor comprises a rotor and stator connected by an alkene moiety that functions as the axle. A and B are DMAP and thiourea catalytic groups, respectively, that can cooperate as Brønsted base and hydrogen bond donor in an organocatalytic conjugate addition. Clockwise rotation (seen from the stator side) of the rotor around the axle, by photochemically and thermally induced steps, controls the position and helical orientation of the catalytic groups A and B, providing sequentially catalysts I, II, and III with different activities and stereoselectivities. The last two isomerization steps [steps 3 and 4 of the 360° rotary cycle (see Fig. 2)] reset the catalyst to its initial stage I.



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(Fig. 2J) was used as a catalyst, racemic (*R,S*)-thiol adduct **4** (Fig. 3C) was obtained in a slow reaction (7% yield after 15 hours). The low activity of the *trans* isomer of motor **1** is attributed to the unfavorable orientation of the DMAP and thiourea moieties on the motor scaffold, which precludes the operation of cooperative bifunctional catalysis. In sharp contrast, after photoisomerization, the Michael addition proceeds significantly faster as the (*M,M*)-*cis*-**1** isomer [stable under the reaction conditions without noticeable isomerization; half-time ($t^{1/2}$) = 120 days at 0°C] provides adduct **4** in 50% yield under the same conditions.

More remarkable is the selective formation of the (*S*) enantiomer of adduct **4** with an e.r. of 75/25 (*S/R*) (Fig. 3D). The next isomer (*P,P*)-*cis*-**1** accelerates the addition reaction even more (83% yield in 15 hours) and exhibits an inversion in enantioselectivity, providing the (*R*) enantiomer of **4** with an e.r. of 23/77 (*S/R*) (Fig. 3E). The relative rates of product formation for the three catalysts (*P,P*)-*trans*-**1**, (*M,M*)-*cis*-**1**, and (*P,P*)-*cis*-**1** are displayed in Fig. 3B (see also fig. S9). The asymmetric Michael addition can be switched on in situ by irradiating the catalyst (*P,P*)-*trans*-**1** at 312 nm [to form (*M,M*)-*cis*-**1**] in the presence

of reactants **2** and **3**, and a significant rate enhancement (40% yield in 15 hours) and comparable stereocontrol (e.r., 74/26, *S/R*) are observed (see fig. S10).

From these data, it is evident that the *cis* isomers of motor **1** display a strongly enhanced catalytic activity compared with *trans*-**1**. Stereocontrol is not observed at all for the *trans* isomer of motor **1**, in contrast to the *cis* isomers. This molecular motor-based organocatalyst has the distinct property that photoisomerization can be used to modulate both catalytic activity and the enantioselectivity. Based on mechanistic studies

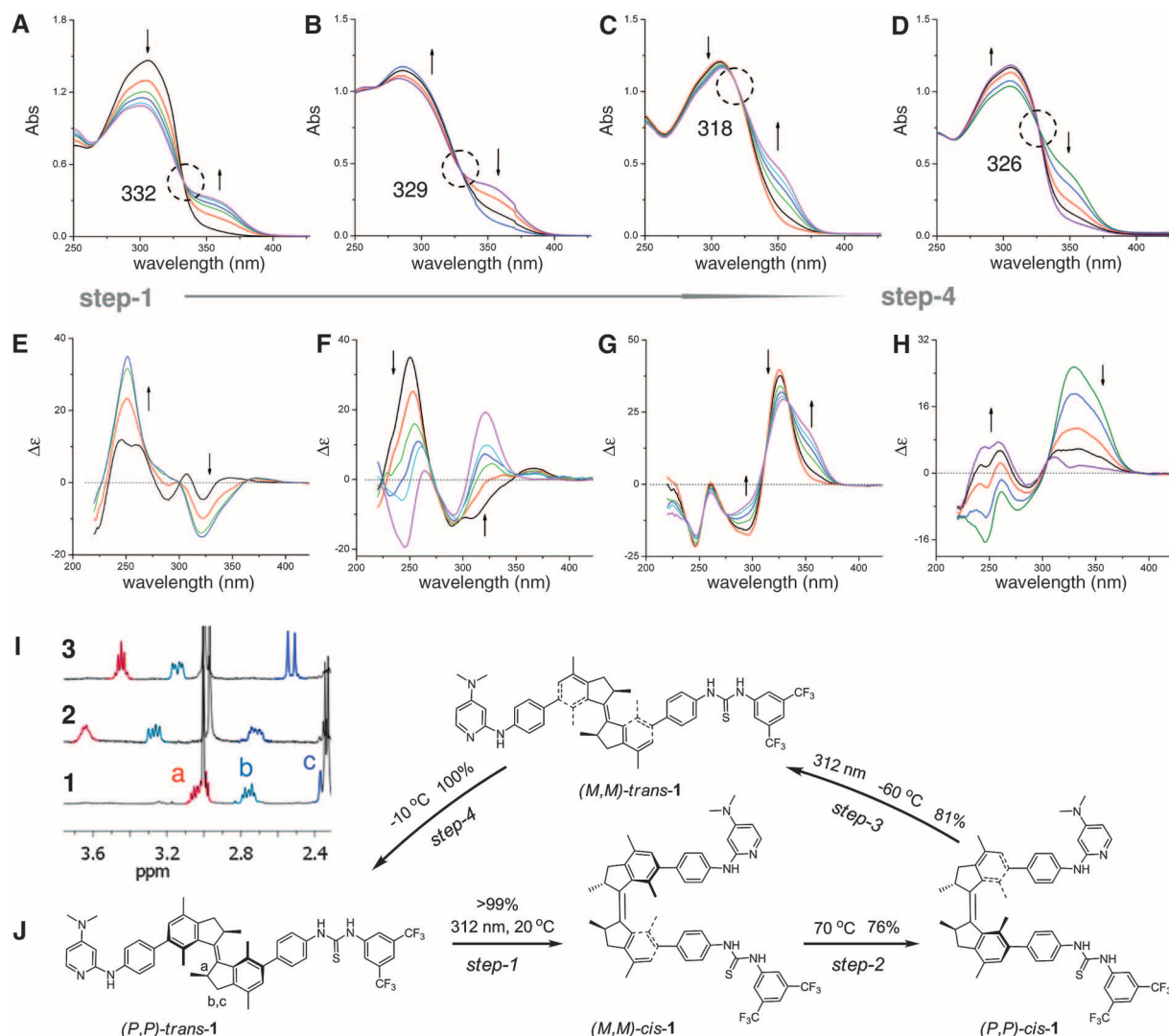


Fig. 2. UV-vis (A to D) and CD (E to H) spectral changes of compound **1** (2.0×10^{-5} M) in each isomerization process of the four-step unidirectional rotary cycle (J) fueled by UV irradiation and thermal energy. (A) UV-vis spectral changes during step 1 upon irradiation at 312 nm ($\Phi^{iso} = 0.37$) in THF at 20°C; isosbestic points are indicated with circles. (B) UV-vis spectral changes during step 2 on heating in THF/isopropanol at 70°C. (C) UV-vis spectral changes during step 3 on irradiation at 312 nm ($\Phi^{iso} = 0.18$) in THF at -60°C. (D) UV-vis spectral changes during step 4 ($t^{1/2} = 57$ min) in THF at -10°C. (E to H) The corresponding CD spectral changes during each of the four isomerization steps, respectively. Spatial orientation of the catalytic groups is controlled by the directional rotary motion of the molecular motor (J). Step 1:

Starting from (*P,P*)-*trans*-**1**; a photoisomerization ($\lambda^{irr} = 312$ nm) provides (*M,M*)-*cis*-**1** in which helix inversion (from *P* to *M*) has occurred and the two catalytic groups are brought into close proximity. Step 2: A thermal helix inversion (70°C) provides (*P,P*)-*cis*-**1**, and the two catalytic units remain in close proximity while a helix inversion from *M* to *P* takes place. Steps 3 and 4: A photochemical step followed by thermal isomerization reset the original structure (*P,P*)-*trans*-**1** via the intermediacy of isomer (*M,M*)-*trans*-**1**, which was not studied as a catalyst due to low thermal stability. The partial $^1\text{H-NMR}$ (CD_2Cl_2) spectra of compounds (*P,P*)-*trans*-**1**, (*M,M*)-*cis*-**1**, and (*P,P*)-*cis*-**1** are shown in (I) **1** to **3**, respectively [for the assignment of the protons, see (I), (*P,P*)-*trans*-**1**; singlet at 3.0 parts per million represents NMe_2 protons].

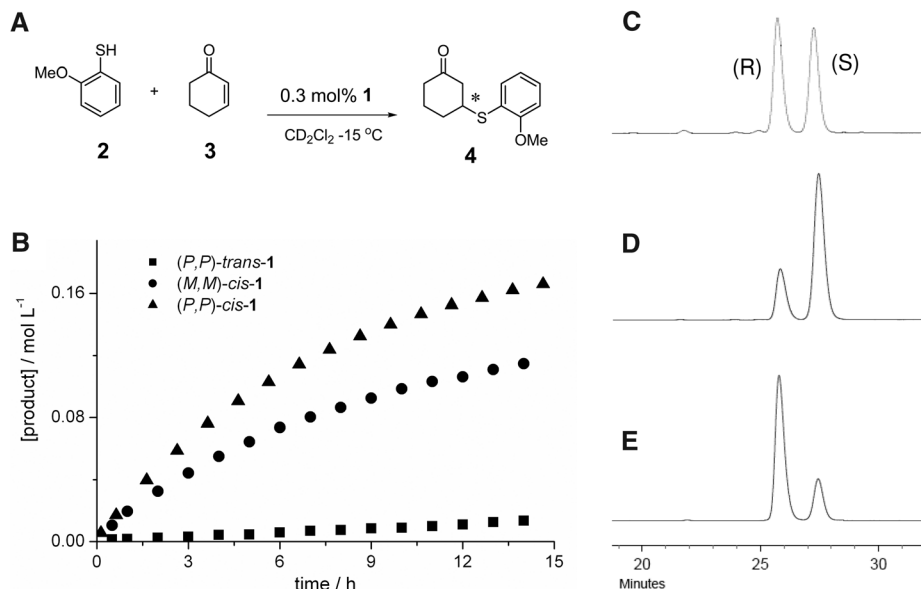


Fig. 3. Catalytic performance of compound **1** for the Michael addition of **2** (0.2 M) to **3** (0.2 M) to give chiral adduct **4**. (A) Reaction scheme and conditions. (B) Reaction kinetics followed by measuring product formation with in situ ^1H -NMR spectroscopy. (C to E) Chiral HPLC traces of the reaction product **4** using catalyst (*P,P*)-*trans*-**1** (e.r., *S/R*, 49/51), (*M,M*)-*cis*-**1** (e.r., *S/R*, 75/25), and (*P,P*)-*cis*-**1** (e.r., *S/R*, 23/77), respectively.

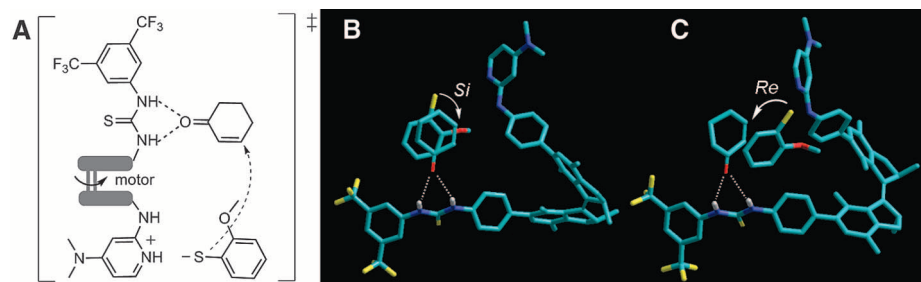


Fig. 4. Proposed ternary complex (A) involved in the mechanism of thiol addition to enone catalyzed by **1** and the energy-minimized structures for the asymmetric Michael addition (B and C) obtained using Hyperchem 8.0 (RM1). (B) Catalyst (*2R,2'R*)-(*M,M*)-*cis*-**1**; thiol addition to the Si face is favored (marked with arrow) to give the product (*S*)-**4**. (C) Catalyst (*2R,2'R*)-(*P,P*)-*cis*-**1**; thiol addition to the Re face is favored to give the product (*R*)-**4**. In the *trans* isomer (not shown), the catalytic units are pointed antiparallel to each other, precluding bifunctional activation (see fig. S11).

of bifunctional organocatalysts for thiol addition (10, 17, 18), it is proposed that this transformation (Fig. 4A) involves an activation of the enone through hydrogen bonding with the thiourea and deprotonation of the thiol nucleophile ($\text{p}K_{\text{a}} = 6.6$) by DMAP ($\text{p}K_{\text{a}} = 9.7$). The experimental observations suggest that the motor rotation not only controls the chirality of the entire system but also gears the catalytically active units to cooperate. In the *trans* isomer, due to the distant orientation of the thiourea and DMAP groups, no cooperative effect is possible, resulting in low catalytic activity. Basic molecular modeling studies were performed to rationalize the reversal in absolute stereochemistry of product **4** on shifting from catalyst (*M,M*)-*cis*-**1** to (*P,P*)-*cis*-**1** (Fig. 4, B and C).

The energy-minimized structure (see note S5) of the ternary complex comprising catalyst (*2R,2'R*)-(*M,M*)-*cis*-**1**, thiol **2**, and enone **3** (Fig.

4B) shows that thiol addition to the Si face of cyclohexenone is favored to give product **4** with the (*S*) configuration in accordance with experimental observation. Addition to the Re face is hampered by severe steric hindrance along the preferred Bürgi-Dunitz trajectory for conjugate addition (19). In contrast, when the (*2R,2'R*)-(*P,P*)-*cis*-**1** catalyst is employed (Fig. 4C), thiol addition to the Re face is more favorable, yielding product **4** with the (*R*)-absolute configuration.

It should be noted that the helicity (*P* or *M*) and directionality (clockwise or counterclockwise) of rotation of the molecular motor is governed by the chirality of the stereogenic centers (*R* or *S*) in the motor unit. The helicity (*P* or *M*) of the motor in turn dictates the spatial orientation of the catalytic groups and, as a consequence, the configuration (*R* or *S*) of the newly formed stereogenic center in the product **4** of the enantioselective catalytic

event. This interdependence implies that on starting with (*2R,2'R*)-(*P,P*)-*trans*-**1** (Fig. 2), a clockwise rotary cycle provides sequentially racemic (*R,S*)-, (*S*)-, and (*R*)-product **4** (Fig. 3, C to E) before returning to produce racemic product after a full rotary cycle.

To further explore this delicate stereochemical interplay, the enantiomer of molecular motor **1**, with the (*2S,2'S*) configuration at the stereogenic centers, was examined. Because the (*2S,2'S*)-(*M,M*)-*trans*-**1** motor is now the starting point, a counterclockwise rotary cycle is induced upon photochemical and thermal isomerization, providing sequentially racemic (*R,S*)-, (*R*)-, and (*S*)-product **4** before returning to produce racemic product after a full rotary cycle (see fig. S12).

Coupling of unidirectional switching to catalytic function, as demonstrated here, may prove to be a key design tool in the construction of future catalysts that can perform multiple tasks in a sequential manner.

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Second-Order Selection for Evolvability in a Large *Escherichia coli* Population

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In theory, competition between asexual lineages can lead to second-order selection for greater evolutionary potential. To test this hypothesis, we revived a frozen population of *Escherichia coli* from a long-term evolution experiment and compared the fitness and ultimate fates of four genetically distinct clones. Surprisingly, two clones with beneficial mutations that would eventually take over the population had significantly lower competitive fitness than two clones with mutations that later went extinct. By replaying evolution many times from these clones, we showed that the eventual winners likely prevailed because they had greater potential for further adaptation. Genetic interactions that reduce the benefit of certain regulatory mutations in the eventual losers appear to explain, at least in part, why they were outcompeted.

Organisms may vary not only in traits that determine their immediate fitness, but also in their potential to generate better-adapted descendants with new beneficial mutations. Evolutionary potential, or evolvability, can be operationally defined as the expected degree to which a lineage beginning from a particular genotype will increase in fitness after evolving for a certain time in a particular environment (1). Evolvability thus reflects a complex probabilistic integration of accessible paths in the fitness landscape influenced by mutation rates, population structure, and epistatic interactions between mutations (2–4). Experiments with microorganisms have shown that genotypes with elevated mutation rates have greater evolvability under certain conditions (5, 6). The evolutionary potential of microorganisms can also vary when the same mutations have different fitness effects in different genetic backgrounds due to epistatic interactions (7–9). The extent to which material differences in evolvability of this latter kind—reflecting genetic architecture (10), rather than mutation rates—spontaneously arise between lineages within asexual populations and play a role in ongoing evolutionary dynamics is unknown (11, 12).

We found that several genetically distinct subpopulations were already present in a 500-generation sample archived from a now >50,000-generation long-term evolution experiment with the bacterium *Escherichia coli* (13, 14). In particular, we characterized numerous clones sampled at 500, 1000, and 1500 generations for the presence of five pre-

viously discovered beneficial mutations (14–18). Specific mutations in the *rbs* operon and *topA*, *spoT*, and *glmUS* genes fixed in the evolving population between 1000 and 1500 generations, and in *pykF* after 1500 generations (14). Two beneficial mutations—the ones affecting *rbs* and *topA*—were already present in many clones sampled at 500 generations (table S1). Thus, we refer to *topA* *rbs* genotypes sampled at generation 500 as eventual winners (EWs) and to other contemporaneous genotypes as eventual losers (ELs). These genotypes may have other beneficial mutations in these same or other genes, and most such mutations could not have been detected by the assays developed for the known mutations.

We further characterized two clonal isolates of each type: EW1 and EW2, each with the *topA* and *rbs* mutations, EL1 with no known mutations, and EL2 with another mutation we call the *rbsI* mutation. The EW *topA* allele is an amino acid substitution in DNA topoisomerase I that alters chromosomal supercoiling, affects the transcription of many genes, and confers a fitness benefit of ~13% when moved into the ancestral genetic background (16). The *rbs* mutations are deletions of different sizes in the ribose utilization operon that occur with high frequency and cause 1 to 2% fitness gains (15). Competition experiments against the ancestral strain showed that these four representative clones were 13 to 23% more fit than the ancestor (Fig. 1). Thus, the ELs, and possibly also the EWs, had other beneficial mutations, in addition to those that could be identified by our initial genotyping.

One would naively expect that the EWs were better adapted than the ELs at 500 generations, but the opposite was true (Fig. 1). Direct competition experiments also showed that the two representative EW clones were at a significant fitness disadvantage relative to the two representative EL clones (fig. S1). In fact, if the fitness deficit of the EWs (–6.3%) had remained constant, they would have gone extinct in another ~350 generations (19). We found no evidence of negative frequency-dependent interactions (20) between EW and EL strains that might have stabilized their continued coexistence in the long-term population (19).

How did descendants of the EWs prevail over EL lineages despite their fitness deficit? The EW-derived lineage may have simply been “lucky” in this one instance of evolution; that is, they might have stochastically gained highly beneficial mutations that allowed them to overtake the EL subpopulations before they were driven extinct. Alternatively, the EW genotypes may have had a greater potential for further adaptation, such that they would reproducibly give rise to higher-fitness descendants and outcompete EL lineages before they were lost. To distinguish between these two hypotheses, we “replayed” evolution by initiating 10 replicate experimental populations from each clone isolated at 500 generations (EW1, EW2, EL1, and EL2). Each population was propagated independently under the same conditions as were used in the long-term evolution experiment for 883 generations, approximately as long as ELs and EWs coexisted in the original population.

To follow the evolutionary dynamics in more detail, we conducted these evolution experiments in a neutral marker divergence format (9, 21, 22). A variant of each of the four *E. coli* test strains was constructed wherein the state of a readily scored phenotypic marker (Ara), which has no effect on fitness under these culture conditions, was altered by a specific point mutation (13). Each experimental population was then started by mixing approximately equal numbers of the original test strain (Ara[–]) and the strain with the changed marker (Ara⁺), with each type grown separately from a single colony to ensure that there was essentially no initial genetic variation within a population and no shared history between independent replicates. Tracking the frequency of this genetic marker in these 40 populations over time allowed us to visually follow and quantitatively analyze the first beneficial mutations that swept to high frequency within each population.

The trajectory for the Ara[–]/Ara⁺ ratio in each population eventually diverged from its starting level, as bacteria with beneficial mutations linked to one marker state arose and outcompeted their ancestors and competitors with less-beneficial

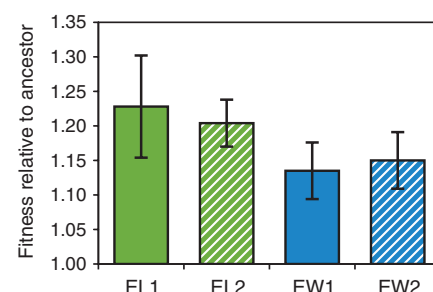


Fig. 1. Fitness of two EW and two EL clones relative to the ancestor of the *E. coli* long-term evolution experiment. Error bars are 95% confidence intervals. All four clones were significantly more fit than the ancestor. Surprisingly, the EL clones were more fit than the EW clones, both as shown here and in direct competition with one another (fig. S1).

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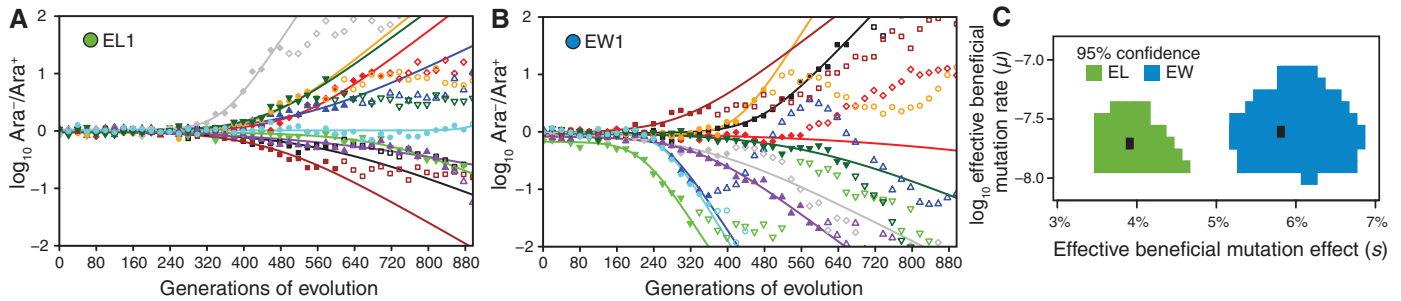


Fig. 2. Replay evolution experiments to measure the evolvability of the four representative 500-generation clones. **(A and B)** The frequencies of Ara⁻ and Ara⁺ versions of each test strain, initially mixed equally, were recorded at regular intervals (symbols) during 883 generations of evolution under the same conditions as were used in the long-term experiment. Marker trajectories for the replay populations initiated from EL1 and EW1 clones are shown (10 replicates each). Shifts in the Ara⁻/Ara⁺ ratio occur when new beneficial mutations linked to one background arise and increase in frequency within the population. Fitting the replicate marker trajectories (lines and solid symbols)

until they deviate significantly from an exponential model (open symbols) provides a distribution of empirical shape parameters for the initial divergence. **(C)** Effective mutation rates (μ) and fitness effects (s) for the first beneficial mutations to sweep to high frequency in a given genetic background were inferred by comparing experimental divergence parameters with those from simulated marker trajectories. Black rectangles represent maximum-likelihood estimates. Representative EW and EL isolates were grouped for this analysis (19). Figure S2 shows data for EL2 and EW2 populations and other steps in the statistical analysis.

mutations (Fig. 2, A and B, and fig. S2, A and B). The shape of the initial divergence of the family of curves generated from evolutionary replicates of the same clone reflects its local fitness landscape. In particular, a simple model that assumes one category of beneficial mutation, with an effective mutation rate (μ) and fitness benefit (s), reproduces the salient features of these dynamics (21), provided that it includes competition between lineages with alternative beneficial mutations, i.e., clonal interference (2, 23, 24).

By performing population genetic simulations to generate families of curves for many μ and s values (19), we determined the parameter combinations that agreed best with the experimental curves (Fig. 2C). The replicate EW marker ratio trajectories diverged earlier and more steeply than the EL trajectories, and the effective size of the first beneficial mutations to sweep to high frequency was significantly larger for the EWs. However, by itself, this first mutation was not sufficient for the EWs to overtake the ELs. From the 6.3% initial fitness deficit of the EWs and the 1.8% larger effect size of their first beneficial mutations, we calculate that, on average, the EWs would still be ~4.5% less fit than the ELs after the first adaptive step for each type (Fig. 3).

To compare evolvability on a time scale that allows a lineage to accumulate multiple beneficial mutations, we isolated a random clone from each replicate population at the 883-generation endpoint. This evolved clone was either Ara⁻ or Ara⁺. We performed head-to-head competitions of every EL-EW pair with opposite Ara marker states to determine their relative fitness (fig. S3). We found that, on this time scale, the EWs overcame their initial fitness deficit and evolved to higher fitness than the ELs by ~2.1%, on average (Fig. 3). Thus, the EWs evidently prevailed in the original long-term population because they had greater evolvability. On average, they achieved higher fitness than the ELs after each type had equal time to evolve by multiple beneficial-mutation steps from its starting point in the fitness landscape. We stress,

however, that this result is necessarily probabilistic in nature. Not every evolved EW clone was able to outcompete every evolved EL clone.

What is the genetic basis of this difference in evolvability? There are three possibilities. First, the EW genetic background may have interacted more favorably with certain potential beneficial mutations than the ancestral background with those same mutations (positive epistasis), thereby opening up additional pathways for adaptive evolution. Conversely, mutations in the ELs may have reduced the effects of otherwise beneficial mutations (negative epistasis), thereby closing off some pathways for adaptation. Finally, a mutation in the EWs may have caused an elevated mutation rate relative to that of the ELs that would allow the EWs to access rarer, more beneficial mutations.

To distinguish the salient genetic differences between the EWs and ELs, we resequenced the genomes of eight evolved *E. coli* isolates from generation 883 of the replay experiments (19). We chose two strains descended from each of the four 500-generation clones, so that we could reconstruct what mutations were present in the original isolates as well as sample mutations that occurred in their descendants (Fig. 4A). We found that the EWs shared only the two known mutations (*topA* and *rbs*) and that both ELs had two previously unknown base substitutions (*topA1* and *fadR*). The EL *topA1* mutation alters the amino acid (isoleucine-34 to serine) directly adjacent to the one changed by the EW *topA* allele (histidine-33 to tyrosine). *FadR* is a regulator of fatty acid and acetate metabolism (25), and the effects of this EL mutation are unknown.

From two to five mutations accumulated during the 883-generation replay experiment in the eight independently evolved isolates (tables S2 to S9). There was no evidence that EWs had an elevated mutation rate that might have contributed to their greater evolvability. The number of replay-phase mutations in the four evolved EWs (16 total) was essentially identical to that in the four evolved ELs (15 total). The only mutation in the original

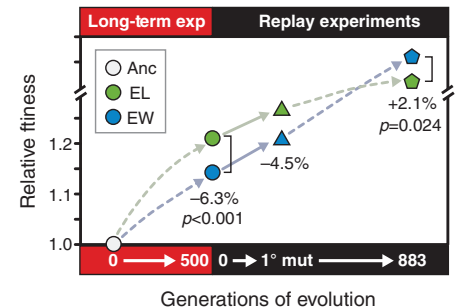
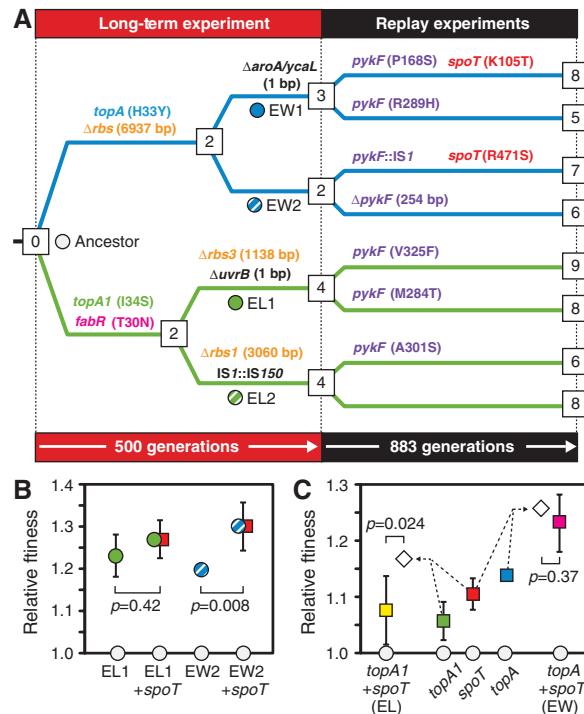


Fig. 3. Greater evolvability of EWs allows them to reproducibly overtake ELs. Two representative EW clones from generation 500 of the long-term evolution experiment were initially at a significant fitness disadvantage relative to two contemporary EL clones (circles). The EWs were somewhat closer in fitness to the ELs, but still lagged behind on average, after the first beneficial mutations swept to high frequency in the replay evolution experiments (triangles), as determined by the marker trajectory divergence analysis. After 883 generations, the representative EWs evolved to a higher fitness on average than the ELs in the replay populations (pentagons). Percentage differences in fitness are for pooled EWs versus ELs at the highlighted time point, and P -values indicate whether this difference was significant (19). Arrows represent presumptive mutational steps, with dashes indicating that the exact number of mutations may vary. The y axis is unlabeled for the final 883-generation replay isolates because their fitness was measured with respect to each other, not relative to the ancestor.

500-generation clones in a gene related to DNA repair or replication (*uvrB*) was shared by the EL1-derived strains, contrary to the expectation if a change in mutation rate drove the difference in evolvability.

It is very likely that most of the 31 observed mutations that occurred during the replay experiments are beneficial. The rate of genomic change due to adaptive evolution greatly exceeds the rate of neutral drift in this system, and mutations in

Fig. 4. (A) Mutations identified by whole-genome resequencing of endpoint *E. coli* clones from the replay evolution experiments and inferred by parsimony in their EW and EL ancestors. Numbers in squares indicate how many mutations accumulated relative to the original long-term ancestor with mutations in key genes labeled. See tables S2 to S9 for complete lists of all mutations. **(B)** Fitness effects of adding the *spoT* mutation that fixed during the long-term experiment to the EL1 and EW2 genetic backgrounds, measured relative to the ancestral strain. Error bars are 95% confidence limits (hidden by symbols in some cases). *P*-values indicate the significance of the hypothesis that addition of the *spoT* mutation caused a fitness difference. **(C)** Fitness effects of the long-term *spoT*, EW *topA*, and EL *topA1* mutations alone and in combination in the ancestral genetic background. Dashed lines converging on empty diamonds show the fitness predicted for each *spoT* and *topA* allele combination given independent multiplicative effects. *P*-values are for the hypothesis of no epistatic interactions under a multiplicative model (31). Error bars are 95% confidence limits.



some of the same genes, operons, and pathways have been found in other isolates from the long-term experiment (14, 26). Two genes independently evolved mutations in more than one of the sequenced 883-generation EL- and EW-derived strains (Fig. 4A). Seven of the eight sequenced clones from the replays evolved mutations in *pykF*, which encodes the metabolic enzyme pyruvate kinase. In the original long-term population, *pykF* mutations were detected in EWs by 1500 generations (table S1) and in *rbs1* ELs by 1000 generations (19). The long-term EW *pykF* mutation is highly beneficial in this environment (14), and all 12 long-term populations substituted *pykF* mutations by 20,000 generations (27). Two of the sequenced replay clones had point mutations in *spoT*, which encodes a bifunctional (p)ppGpp synthesis and degradation enzyme that is a global regulator of gene expression. The next mutation found in the long-term EW lineage, after *rbs* and *topA*, was also a *spoT* base substitution (table S1). This *spoT* mutation has been shown to affect the transcription of numerous genes and to confer a fitness benefit of ~9% when moved into the ancestral strain background (17, 28).

We observed *pykF* mutations in both EW- and EL-derived strains. However, both *spoT* mutations arose in EW-derived lines (Fig. 4A). This association with genetic background raised the possibility that *spoT* mutations might be involved in epistatic interactions that underlie differences in EW-EL evolvability, especially given the potential for widespread pleiotropic effects caused by mutations in this global regulator (29). To increase the statistical power for detecting an association, we

sequenced the complete *spoT* reading frame in all 40 EW and EL endpoint clones isolated from the replay experiments. We found *spoT* mutations in 6 of the 20 evolved EW clones (table S10). Notably, we found no *spoT* mutations in the 20 EL clones, a difference unlikely by chance (two-tailed Fisher's exact test, *P* = 0.0202).

To test directly for epistatic interactions, we measured the fitness effects of adding the *spoT* allele that arose during the long-term experiment to the EW2 and EL1 strain backgrounds. We found that this *spoT* allele confers a large fitness benefit in the EW background, but has no significant effect in the EL background (Fig. 4B). Because changes in chromosomal supercoiling also may have widespread pleiotropic effects, it seemed likely that interactions with the *topA* alleles specific to the EW and EL backgrounds might explain these epistatic effects. To test this hypothesis, we constructed otherwise isogenic strains carrying only the EW *topA* or EL *topA1* alleles on the ancestral background and then measured the fitness effects of adding the *spoT* mutation that arose during the long-term experiment to each strain (Fig. 4C). The EL *topA1* allele is beneficial on its own, though less so than the EW *topA* mutation. The *spoT* mutation is also highly beneficial on its own and has essentially the same fitness effect in the presence of the EW *topA* allele. By contrast, this *spoT* mutation is neutral, or at least much less beneficial, in combination with the EL *topA1* mutation.

These results therefore support the hypothesis of negative epistasis between EL mutations and later mutations that arise in *spoT*, and they contradict the hypothesis of positive epistasis between

the EW and that *spoT* allele. Highly beneficial *spoT* mutations are evidently at the leading edge of the adaptive mutations that are accessible to the EW subpopulation, which is why they often evolve in this background. The alternative *topA1* mutation that arose in the EL subpopulation evidently renders those *spoT* mutations neutral, leaving other less-beneficial mutations the best available. In essence, the ELs followed a trajectory in the fitness landscape that allowed more rapid improvement early on, but which shut the door on at least one important avenue for further improvement. By contrast, the EWs followed a path that did not preclude this option, giving them a better than otherwise expected chance of overtaking the ELs. Because *spoT* mutations evolved in only 6 of the 20 EW replays, and because it took multiple mutations for the EWs to overtake the ELs, it is likely that epistatic interactions with other beneficial mutations also contributed to their differences in evolutionary potential.

We have demonstrated in detail a case in which epistatic interactions between beneficial mutations caused differences in bacterial evolvability that appear to have played a pivotal role in the evolution of a population. Similar cases are expected in any population of asexual organisms that evolve on a rugged fitness landscape with substantial epistasis, as long as the population is large enough that multiple beneficial mutations accumulate in contending lineages before any one mutation can sweep to fixation (2, 11, 22–24, 30). This scenario thus provides a general mechanism for the evolution of evolvability by “second-order selection” (3) on genetic architecture. Our results also suggest that studying the interactions among regulatory networks could lead to a deeper understanding of how genetic changes in those networks might either promote or impede evolvability at a systems level.

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Supporting Online Material

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Materials and Methods

Figs. S1 to S3

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Independent and Parallel Recruitment of Preexisting Mechanisms Underlying C₄ Photosynthesis

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C₄ photosynthesis allows increased photosynthetic efficiency because carbon dioxide (CO₂) is concentrated around the key enzyme RuBisCO. Leaves of C₄ plants exhibit modified biochemistry, cell biology, and leaf development, but despite this complexity, C₄ photosynthesis has evolved independently in at least 45 lineages of plants. We found that two independent lineages of C₄ plant, whose last common ancestor predates the divergence of monocotyledons and dicotyledons about 180 million years ago, show conserved mechanisms controlling the expression of genes important for release of CO₂ around RuBisCO in bundle sheath (BS) cells. Orthologous genes from monocotyledonous and dicotyledonous C₃ species also contained conserved regulatory elements that conferred BS specificity when placed into C₄ species. We conclude that these conserved functional genetic elements likely facilitated the repeated evolution of C₄ photosynthesis.

Plants use CO₂ as a substrate to make sugars through a complex biochemical process involving the Sun's energy. The key photosynthesis enzyme RuBisCO (ribulose-1,5-bisphosphate carboxylase-oxygenase) fixes CO₂ into a three-carbon molecule, but it does not completely distinguish between O₂ and CO₂ (1). Because of this, at least 45 lineages from 19 families of flowering plants have evolved C₄ carbon-concentrating mechanisms that enhance the concentration of CO₂ around RuBisCO and increase the efficiency of photosynthesis by reducing the energy wasted when RuBisCO fixes O₂ (2).

All C₄ lineages generate high concentrations of CO₂ around RuBisCO by compartmentalizing photosynthesis, either within or between cells (3, 4), and normally carbon is fixed in mesophyll (M) cells to generate high concentrations of a four-carbon acid. The acid then diffuses through plasmodesmata into adjacent cells, often the cells of the bundle sheath (BS), where decarboxylases release CO₂ around RuBisCO (3). In some cases,

multiple C₄ acid decarboxylases are used to deliver CO₂ to RuBisCO (5, 6). High activities of these C₄ acid decarboxylases around the veins of C₃ species may have facilitated the polyphyletic evolution of C₄ photosynthesis (7, 8).

The compartmentalization of photosynthesis results from substantial differences in gene expression in C₄ leaves relative to those of C₃ species (9, 10). Genes important for C₄ photosynthesis have been recruited from orthologs present in C₃ species, and in many cases this has involved alterations in spatial patterns of expression, restricting or enhancing the accumulation of proteins to specific cell types (11, 12). For example, in most cases, carbonic anhydrase, phosphoenolpyruvate carboxylase, and pyruvate orthophosphate dikinase accumulate preferentially in M cells, whereas a C₄ acid decarboxylase and RuBisCO accumulate in BS cells (13, 14). As genes are recruited into C₄ photosynthesis, alterations to the sequence of C₃ genes can lead to cis-elements that allow accumulation of the cognate protein in M or BS cells (12). Despite large differences in gene expression between M and BS cells of C₄ leaves (9, 10) and the recruitment of some genes belonging to multigene families into the C₄ pathway, no cis-elements have been

identified that regulate multiple genes required for C₄ photosynthesis (15–17).

NAD-dependent malic enzyme (NAD-ME) accumulates in BS cells of NAD-ME-type C₄ plants, where it releases CO₂ around RuBisCO (18). Because NAD-ME is encoded by two genes that generate a heterodimer (8), we used it as a model to understand how gene families are recruited into C₄ photosynthesis. We isolated full-length transcript sequences for two NAD-ME genes from *Cleome gynandra*, which is the C₄ plant most closely related to the model *Arabidopsis thaliana* (18). RNA hybridization in situ showed that *CgNAD-ME1&2* transcripts are specific to BS cells (Fig. 1, A and B). Genomic sequences for the two genes were isolated, and a translational fusion between *CgNAD-ME1* (promoter, exons, and introns) and *uidA* encoding the β-glucuronidase (GUS) reporter was generated. Stable transformants of *C. gynandra* expressing *CgNAD-ME1::uidA* accumulated GUS specifically in BS cells (Fig. 1C). When introns were removed and the *CgNAD-ME1* promoter was replaced by the CaMV 35S promoter that directs expression in both M and BS cells (17, 19), GUS also accumulated specifically in BS cells (Fig. 1D). This result contrasted with the accumulation of GUS in both M and BS cells when the *CgNAD-ME1* gene was not present (Fig. 1E and fig. S1A). These data indicate that sequences in the transcribed region of the gene are sufficient for BS-specific accumulation of *CgNAD-ME1*.

Microprojectile bombardment of *pCaMV 35S::CgNAD-ME1::uidA* generated GUS specifically in BS cells (Fig. 1G and fig. S1B), in contrast to a *CaMV 35S::uidA* control in which M and BS cells accumulated GUS in roughly equal proportions (Fig. 1F). The full-length coding region of *CgNAD-ME2* under control of the CaMV 35S promoter also resulted in BS-specific accumulation of GUS in *C. gynandra* (Fig. 1H). This suggests that for both *CgNAD-ME* genes the same mechanism generates BS-specific accumulation.

A 3' deletion series of *CgNAD-ME1* indicated that 240 nucleotides toward the 5' end were sufficient for BS-specific accumulation of GUS, as was the equivalent region of *CgNAD-ME2* (Fig. 2, A and B, and fig. S1, C to E). This BS specificity was due to defined sequence, because when we

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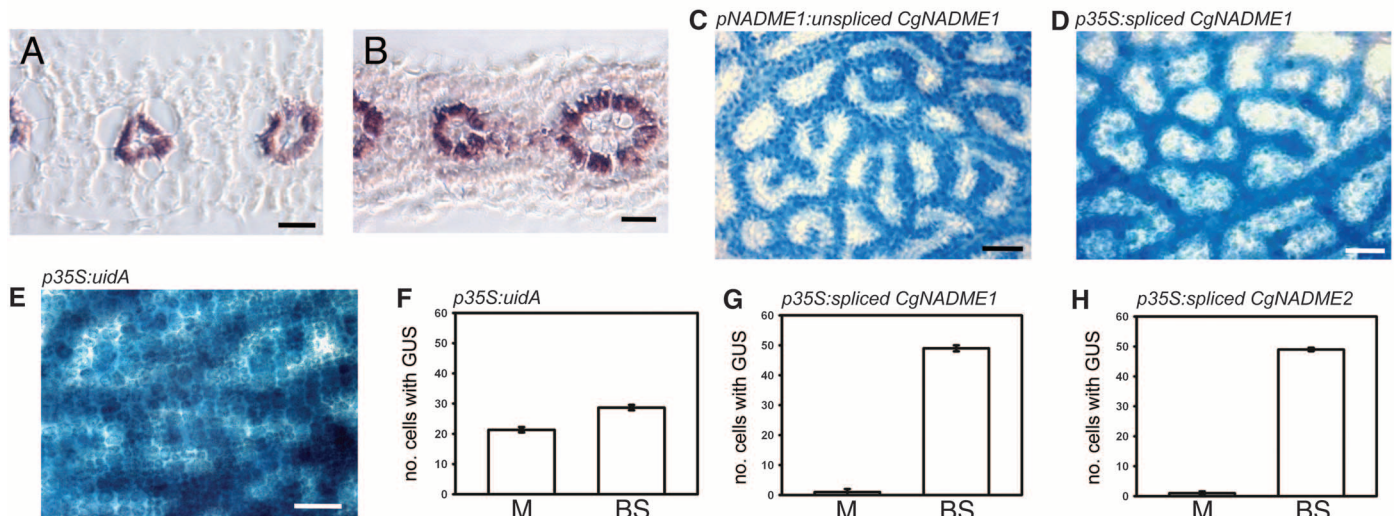


Fig. 1. (A and B) *CgNAD-ME1&2* transcripts in *C. gynandra* BS cells. Scale bars, 50 μ m. (C and D) GUS in the BS of *C. gynandra* transformants containing *uidA* fused to *CgNAD-ME1* under control of the *CgNAD-ME1* promoter (C) or the CaMV 35S promoter (D). (E) Leaf from *C. gynandra* transformant containing *uidA* under control of the CaMV 35S promoter. Scale bars in (C) to (E), 100 μ m. (F to H) *C. gynandra* M and BS cells accumulate GUS after microprojectile bombardment of CaMV 35S driving *uidA* (F) or the spliced coding regions of *CgNAD-ME1* (G) and *CgNAD-ME2* (H). Construct details are shown in fig. S5.

placed 240 nucleotides from the 3' end of the gene in this 5' position, GUS accumulated in both M and BS cells (fig. S1F). Replacing the *CgNAD-ME1* 5' untranslated region (UTR) with that from the CaMV 35S promoter also maintained BS specificity (Fig. 2C), whereas removal of the *CgNAD-ME1* coding region, leaving only the 5' UTR, led to GUS accumulation in both M and BS cells (Fig. 2D). These data indicate that the *CgNAD-ME1* 5' UTR is neither necessary nor sufficient for BS specificity.

Placing the 240-nucleotide fragment upstream of the CaMV 35S promoter resulted in loss of BS specificity (Fig. 2E), indicating that these 240 nucleotides must be present in the transcript; this finding suggested a posttranscriptional mechanism. When the 240-nucleotide fragment was inserted downstream of the predicted stop codon, this also resulted in loss of BS specificity (Fig. 2F). A frameshift construct that maintained nucleotide sequence but translated into different amino acids still conferred BS specificity (fig. S1G). Thus, 240 nucleotides from the coding region of both *CgNAD-ME1&2* genes is sufficient for BS-specific accumulation in *C. gynandra*. This region must be situated in both the transcribed and translated region of the gene, but amino acid sequence is not important. This conclusion contrasts with reports that cis-elements governing cell specificity are present in promoter regions or UTRs (11, 15–17).

Analysis of orthologous *NAD-ME* genes from the closely related *C₃* species *A. thaliana* indicated that nucleotide sequences within the mature coding regions were on average 81% identical to those in *C. gynandra*. We introduced intact *AtNAD-ME* genes fused to *uidA* into *C. gynandra*. Stable transformants of *C. gynandra* expressing *AtNAD-ME1::uidA* accumulated GUS specifically in BS cells (Fig. 3A). Promoters of genes

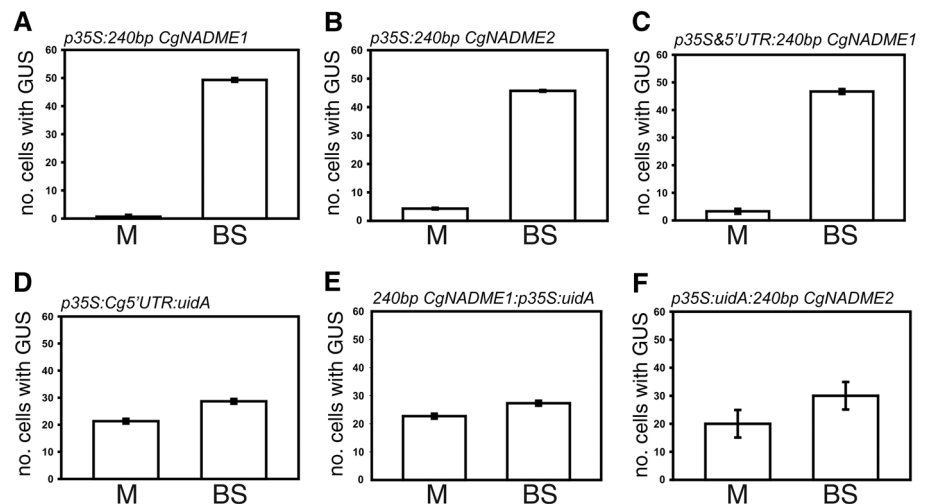


Fig. 2. (A to F) *C. gynandra* M and BS cells accumulating GUS after microprojectile bombardment of 240 base pairs (bp) of *CgNAD-ME1* (A) or *CgNAD-ME2* (B), the CaMV 35S promoter with its own 5' UTR driving expression of 240 bp of *CgNAD-ME1* (C) or of *CgNAD-ME1* 5' UTR-*uidA* alone (D), the 5' 240 bp of *CgNAD-ME1* placed upstream of CaMV 35S (E), or the 5' 240 bp of *CgNAD-ME1* downstream of the *uidA* stop codon (F). Construct details are shown in fig. S6.

from *C₃* species that encode proteins recruited into the *C₄* cycle do not generate M or BS specificity in *C₄* species (20, 21). BS specificity was also observed when either *AtNAD-ME1::uidA* or *AtNAD-ME2::uidA* were used in microprojectile bombardment of *C. gynandra* (Fig. 3, B and C). The 5' UTRs and spliced coding regions of the *A. thaliana* *NAD-ME1&2* genes under control of the CaMV 35S promoter were sufficient to generate GUS accumulation in *C. gynandra* BS cells (Fig. 3, D and E). A 3' deletion series of each of the *AtNAD-ME* coding regions indicated that 240 nucleotides from the 5' end of each led to accumulation of GUS specifically in the BS of *C. gynandra* (Fig. 3, F and G).

In *A. thaliana*, fusions between *uidA* and intact *AtNAD-ME1&2* generated GUS in multiple cell types, including mid-vein, M, and BS cells (Fig. 3, H and I, and fig. S2), and promoter regions are responsible for these patterns (8). Thus, the promoter is sufficient to control spatial patterns of gene expression in *C₃* *A. thaliana*, whereas in *C₄* *C. gynandra*, elements in the coding region (also found in the *C₃* *NAD-ME* genes) have been recruited to recognize one or more trans-factors that generate BS specificity.

Domains of *NAD-ME* genes encoding the mitochondrial *C₄* acid decarboxylase and *NADP-ME* genes encoding the chloroplastic *C₄* acid decarboxylase show conservation among lineages of

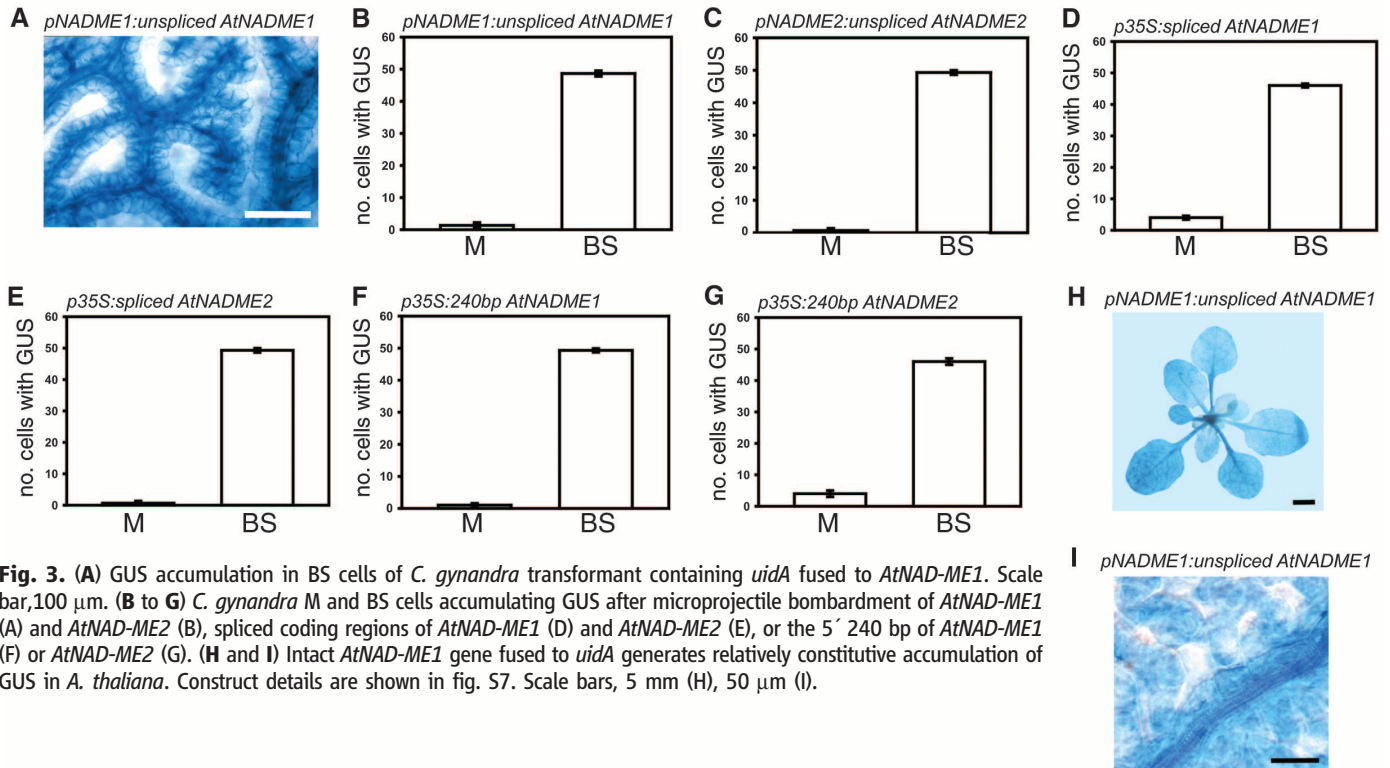


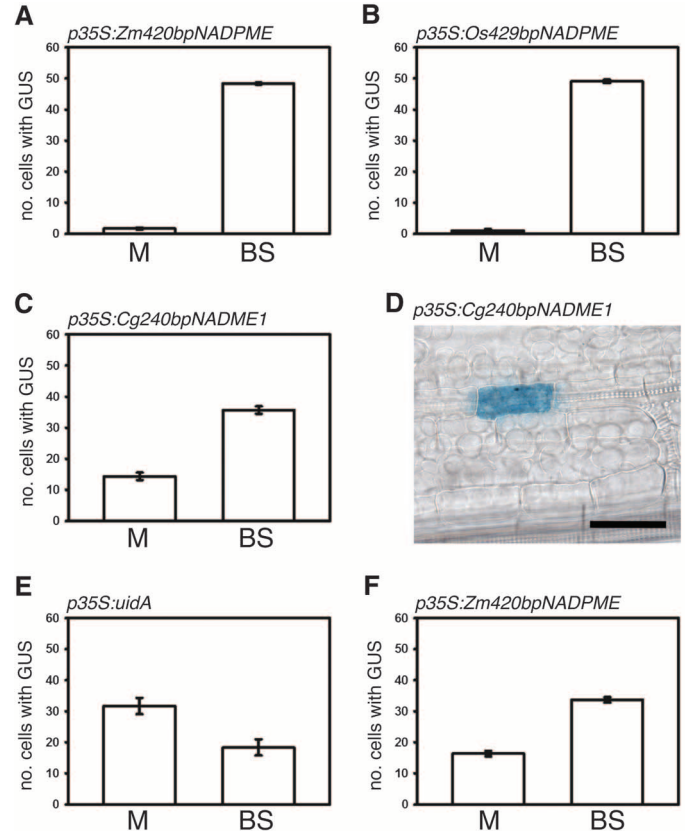
Fig. 3. (A) GUS accumulation in BS cells of *C. gynandra* transformant containing *uidA* fused to *AtNAD-ME1*. Scale bar, 100 μ m. (B to G) *C. gynandra* M and BS cells accumulating GUS after microprojectile bombardment of *AtNAD-ME1* (A) and *AtNAD-ME2* (B), spliced coding regions of *AtNAD-ME1* (D) and *AtNAD-ME2* (E), or the 5' 240 bp of *AtNAD-ME1* (F) or *AtNAD-ME2* (G). (H and I) Intact *AtNAD-ME1* gene fused to *uidA* generates relatively constitutive accumulation of GUS in *A. thaliana*. Construct details are shown in fig. S7. Scale bars, 5 mm (H), 50 μ m (I).

C₄ plants. For example, *NADP-ME* from *C₄* maize has a 420-nucleotide region that is similar to the *CgNAD-ME* genes (fig. S3) (it is longer because the predicted chloroplast transit peptide is longer than that of the mitochondrial targeting sequence of the *C. gynandra* *NAD-ME*). Microprojectile bombardment of this region from *ZmNADP-ME* into *C. gynandra* led to BS-specific accumulation of GUS (Fig. 4A), as did the orthologous rice *NADP-ME* coding sequence (Fig. 4B).

Microprojectile bombardment of maize with the 240-nucleotide sequence from *CgNAD-ME1* or *AtNAD-ME1* fused to *uidA* also produced preferential accumulation of GUS in BS cells (Fig. 4, C and D, and fig. S4A). The *CaMV 35S::uidA* construct generated preferential accumulation of GUS in M cells (Fig. 4E), which likely is associated with suberization of maize BS cell walls inhibiting entry of microprojectiles (19). However, for both *ZmNADP-ME* and *OsNADP-ME* genes, preferential BS expression was observed (Fig. 4F and fig. S4B). Promoter and 5' UTR regions of maize *NADP-ME* generate preferential accumulation of GUS in the BS (22), and so both transcriptional and posttranscriptional mechanisms are likely responsible for *ZmNADP-ME* BS-specific expression.

Our results indicate that these separate lineages of *C₄* plants likely possess functionally equivalent mechanisms that control the accumulation of proteins important for *C₄* photosynthesis in BS cells. Because these sequences are present in *C₃* *Arabidopsis* and rice and are functional within *C₄* *C. gynandra* and maize, we conclude that these genes show a conserved functional latency.

Fig. 4. (A and B) *C. gynandra* M and BS cells accumulating GUS after microprojectile bombardment of 420 bp of maize *ZmNADP-ME* (A) or 429 bp of rice *OsNADP-ME* (B) under control of the *CaMV 35S* promoter. (C) Maize M and BS cells accumulating GUS after microprojectile bombardment of 240 bp of *CgNADME1*. (D) Representative image of maize BS cell accumulating GUS after microprojectile bombardment of 240 bp of *CgNAD-ME1*. Large rectangular BS cells lie adjacent to veins; M cells lacking GUS are circular. Scale bar, 50 μ m. (E) The *CaMV 35S* promoter generates preferential expression of *uidA* in maize M cells after microprojectile bombardment. (F) A 420-bp sequence from *ZmNADP-ME1* is sufficient for preferential accumulation in BS cells of maize. Construct details are shown in fig. S8.



This evolutionary parallelism may be explained by the hypothesis that a number of trans-factors with similar properties (or possibly only one such

factor) are responsible for the accumulation of *NAD-ME* or *NADP-ME* proteins in the BS. This hypothesis is supported by the fact that genes

from C_3 species are recognized by the regulatory circuitry responsible for generating expression in BS cells of multiple C_4 lineages. Our data also indicate the importance of posttranscriptional control of *NAD-ME* genes. Loss of BS specificity when the 240-nucleotide element is present in the transcript but downstream of the stop codon argues against regulation by a small RNA, although it is possible that an RNA binding protein recognizes this 240-nucleotide sequence. Irrespective of the exact mechanism, it is noteworthy that separate lineages of C_4 plants show similar regulation, and so the trans-factors required would have been repeatedly recruited into C_4 photosynthesis.

Our data suggest that cell-specific accumulation of proteins in C_4 leaves can be generated without sequence alterations to genes that encode them. This indicates that genes from C_3 species can be directly recruited into C_4 photosynthesis and implies that an initial step in this process involves modification of trans-factors to make use of existing cis-elements to generate cell specificity. Subsequent regulatory and functional alterations can follow (12, 23). Our data also indicate that cell specificity in the C_4 leaf can be generated by elements in the coding region of genes. Regulation of cell type specificity in the C_4 leaf by conserved elements in coding sequences may be a mechanism that facilitates

the coordinate recruitment of multigene families into the pathway.

These results suggest that engineering C_4 photosynthesis in rice to increase yield may be possible (24, 25). The fact that genes present in C_3 species can be recruited into cell-specific functions in the C_4 pathway without alterations to sequence indicates that the trans-factor(s) responsible for generating cell-specific accumulation of proteins must be identified for potential manipulation in rice.

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Supporting Online Material

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Cardiac Myosin Activation: A Potential Therapeutic Approach for Systolic Heart Failure

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Decreased cardiac contractility is a central feature of systolic heart failure. Existing drugs increase cardiac contractility indirectly through signaling cascades but are limited by their mechanism-related adverse effects. To avoid these limitations, we previously developed omecamtiv mecarbil, a small-molecule, direct activator of cardiac myosin. Here, we show that it binds to the myosin catalytic domain and operates by an allosteric mechanism to increase the transition rate of myosin into the strongly actin-bound force-generating state. Paradoxically, it inhibits adenosine 5'-triphosphate turnover in the absence of actin, which suggests that it stabilizes an actin-bound conformation of myosin. In animal models, omecamtiv mecarbil increases cardiac function by increasing the duration of ejection without changing the rates of contraction. Cardiac myosin activation may provide a new therapeutic approach for systolic heart failure.

Heart failure is a common human disease; after 40 years of age, the lifetime risk of developing heart failure is 20% for both women and men. Despite advances in medical therapy between 1996 and 2006, hospitalizations for heart failure rose by 25% to over 1.1 million

(1). Once hospitalized for heart failure, mortality rates at 30 days, 1 year, and 5 years were as high as 10%, 22%, and 42% in patients from four United States communities (2). In its most common manifestation, heart failure is marked by a decrease in cardiac contractility and called systolic

heart failure (3). To preserve cardiac output, the body increases sympathetic tone and activates neurohormonal pathways. These compensatory mechanisms can, however, accelerate the decline of cardiac systolic function (4).

Current heart failure therapies rely on two different strategies. The most successful is to block neurohormonal activation with inhibitors of the renin-angiotensin pathway, β -adrenergic blockers, and/or aldosterone receptor blockers (4). The second approach is to increase cardiac contractility, which is accomplished indirectly by activating second-messenger signaling pathways that increase cardiac myocyte intracellular calcium concentration (e.g., β -adrenergic receptor agonists or phosphodiesterase inhibitors). Unfortunately, these drugs also increase heart rate and myocardial oxygen consumption and can produce clinically significant arrhythmias and hypotension, which contributes to higher mortality (5).

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We hypothesized that directly activating the contractility of the cardiac sarcomere would improve cardiac performance while avoiding the adverse effects of indirect mechanisms. The sarcomere is made up of interdigitating thin and thick filaments. Myosin, the main component of the thick filament, uses chemical energy derived from ATP hydrolysis to produce force for contraction. Myosin motors act upon thin filaments composed of actin and the troponin-tropomyosin regulatory complex. In resting muscle, the free calcium concentration is low, and the regulatory proteins prevent myosin from interacting with actin. During each heartbeat, calcium is released transiently from the sarcoplasmic reticulum into the cytoplasm, where it binds to troponin and allows myosin to interact with actin filaments and to produce contraction. The muscle relaxes as calcium is removed from the cytoplasm (6).

Conceptually, the direct activation of the cardiac sarcomere can be achieved by either sensitizing the regulatory proteins to calcium or activating cardiac myosin directly. A high-throughput screen of the cardiac myosin adenosine triphosphatase (ATPase) identified a small-molecule activator of cardiac myosin that was further optimized for potency, physical properties, and pharmacokinetics, which culminated in the synthesis of omecamtiv mecarbil (molecular weight 401.43, formerly CK-1827452) (7) (Fig. 1A). When tested in a dog model of heart failure, omecamtiv mecarbil improved cardiac function in the absence of changes in myocardial oxygen consumption (8). We sought to understand the mechanistic basis for this effect on cardiac contractility.

Omecamtiv mecarbil selectively activates the S1 domain of cardiac myosin but not other muscle myosins, as demonstrated by using heterologous reconstituted versions of troponin-tropomyosin-regulated actin-myosin (9). Different myosin S1 isoforms can interact interchangeably with either the cardiac or skeletal muscle thin filaments to hydrolyze ATP (including myosins that are not derived from striated muscle, such as smooth muscle myosin). Omecamtiv mecarbil increased the rate of ATP turnover only when cardiac myosin S1 was present, irrespective of the source of thin filament (troponin-tropomyosin-regulated actin) and not when fast skeletal or smooth muscle myosin were present (Fig. 1B and fig. S1). Further, isothermal titration calorimetry showed that omecamtiv mecarbil binds to purified cardiac myosin S1 with an affinity of $1.6 \pm 0.3 \mu\text{M}$ and a stoichiometry of no more than one molecule of omecamtiv mecarbil per cardiac myosin S1, but showed no significant binding to skeletal or smooth myosin (fig. S2).

The enzymatic and mechanical cycles of myosin are tightly coupled (Fig. 1C) (10, 11), and thus, analysis of the individual kinetic steps in the cardiac myosin ATPase cycle can provide insight into the mechanism of action for omecamtiv mecarbil. The myosin-ATP intermediate binds weakly to the actin filament. In concert with the

hydrolysis of ATP to adenosine diphosphate and inorganic phosphate (ADP-P_i) and subsequent release of P_i from myosin, there is a transition from the weakly actin-bound state to a strongly actin-bound state accompanied by a force-producing power stroke (12). Omecamtiv mecarbil accelerated

the transition rate from the weakly bound to the strongly bound force-producing state as indicated by an increase in the actin-dependent rate of phosphate release from cardiac myosin S1 [median effective concentration (EC₅₀) = $2.3 \pm 0.09 \mu\text{M}$ (SD)] (Fig. 1D and fig. S3) that was

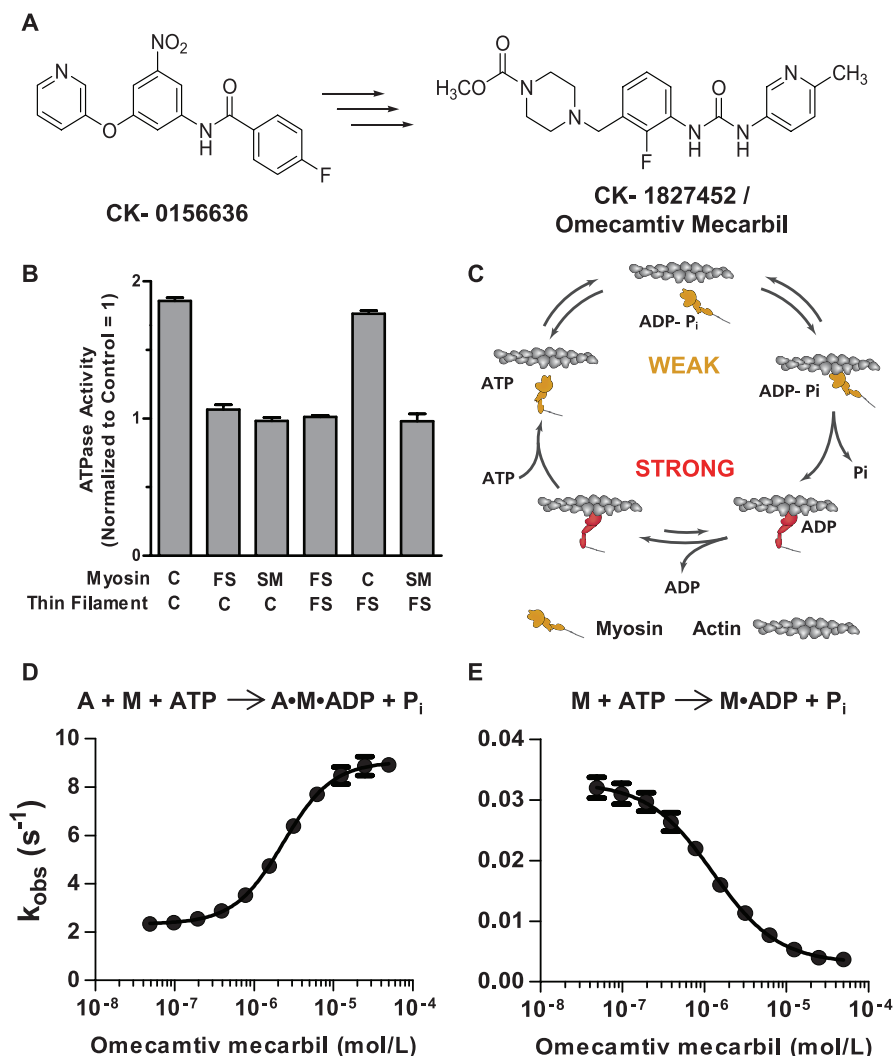


Fig. 1. Identification and characterization of omecamtiv mecarbil. (A) The chemical structure of the original hit, CK-0156636, and the chemical structure of omecamtiv mecarbil, whose optimization and synthesis have been previously reported (7). (B) Target identification of omecamtiv mecarbil using heterologous reconstituted combinations of the troponin-tropomyosin regulated actin-myosin system. Means $\pm$ SD are shown for three to five replicates at each condition. ATPase measurements were made at 25°C in 12 mM Pipes, 2 mM MgCl₂, 1 mM dithiothreitol at pH 6.8 and pCa 6.75 by using a coupled enzyme system consisting of pyruvate kinase and lactate dehydrogenase and monitoring the oxidation of the reduced form of nicotinamide adenine dinucleotide (NADH) at 340 nM (14). C, FS, and SM denote cardiac, fast skeletal, and smooth muscle myosin isoforms. Similarly, C and FS denote reconstituted cardiac and fast skeletal thin filaments, respectively. (C) The mechanochemical cycle of myosin. Yellow indicates myosin weakly bound to actin and red indicates myosin strongly bound to actin [adapted from an illustration, courtesy of J. Spudich, Stanford University]. The effect of omecamtiv mecarbil on the phosphate release rate is shown for actin (A) plus myosin (M) in panel (D) and of myosin alone in panel (E). Transient-state kinetic measurements of phosphate release from cardiac myosin S1 ($n = 3$ for each data point, means $\pm$ SD) were carried out by rapidly mixing a solution of myosin and ATP with 7-diethylamino-3-[(2-maleimidyl)ethyl]amino carbonyl coumarin—modified phosphate-binding protein (MDCC-PBP) plus or minus actin with a stopped-flow apparatus under single-turnover conditions at 25°C. Phosphate released from myosin binds rapidly to MDCC-PBP, which leads to an increase in its fluorescence (13). The rate of phosphate release is measured at various concentrations of omecamtiv mecarbil to construct a dose response.

Fig. 2. The proposed binding site for omecamtiv mecarbil to cardiac myosin S1. The ribbon diagram to the left shows the major features of the myosin S1 head. A space-filling model of the myosin structure showing the position of the identified peptide is shown to the right (the structure of the chicken skeletal S1 fragment, PDB ID 2MYS, was used as a model because the cardiac S1 structure has not been determined). The compound was manually fit into the cleft containing the identified labeled peptide. The red residue indicates the labeled amino acid serine 148.

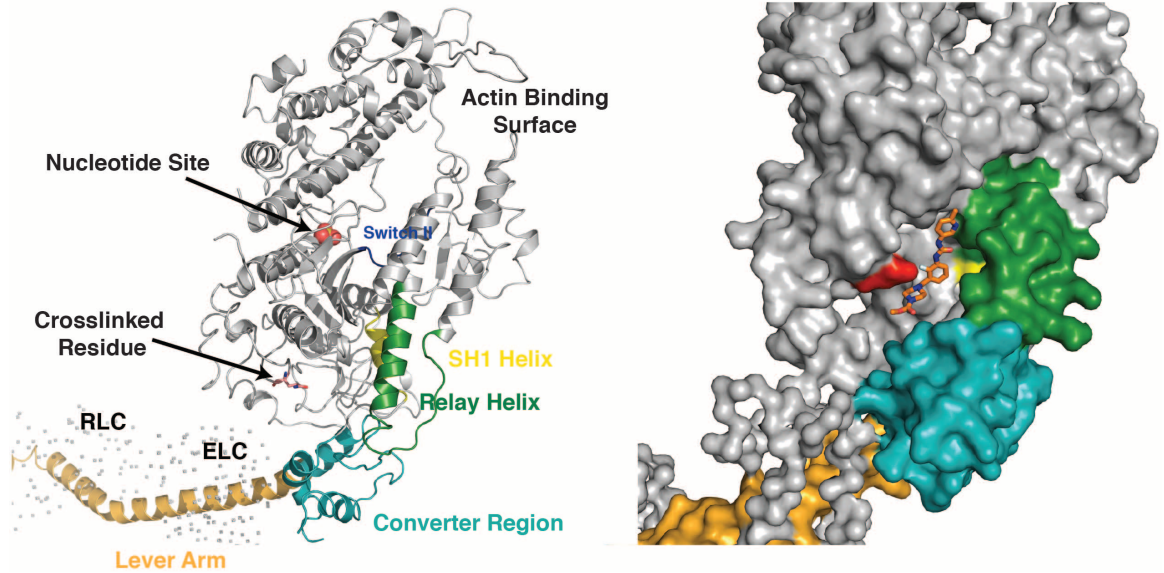


Table 1. The effects of omecamtiv mecarbil, isoproterenol (ISO), and their combination on cardiac myocyte contractility and calcium transient. The effects of omecamtiv mecarbil, isoproterenol, and the combination of the two drugs on FS, the Fura-2 ratio at the peak of contraction (systole), during relaxation (diastole), and the time to 75% decline from peak systolic calcium (T75% Ca^{2+}). Data are means $\pm$ SEM; statistics were performed using a one-way ANOVA with a post hoc Student-Newman-Keuls test.

Treatment	n	FS (% baseline)	Fura-2 ratio		T75% Ca^{2+} (s)
			Systolic	Diastolic	
Control	6	100 $\pm$ 7.7	1.23 $\pm$ 0.017	0.80 $\pm$ 0.005	0.32 $\pm$ 0.018
OM (200 nM)	6	146 $\pm$ 3.4*	1.19 $\pm$ 0.015	0.78 $\pm$ 0.004	0.33 $\pm$ 0.025
ISO (2 nM)	6	169 $\pm$ 13*	1.38 $\pm$ 0.017*†	0.84 $\pm$ 0.011*†	0.25 $\pm$ 0.009*†
OM (200 nM) + ISO (2 nM)	6	212 $\pm$ 15*†‡	1.37 $\pm$ 0.013*†	0.84 $\pm$ 0.015*†	0.25 $\pm$ 0.011*†

$P < 0.05$: *Compared with control, †Compared with OM, ‡Compared with isoproterenol.

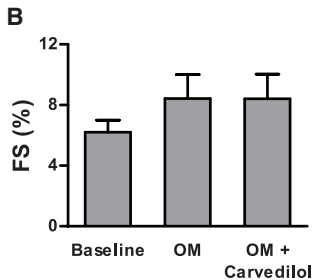
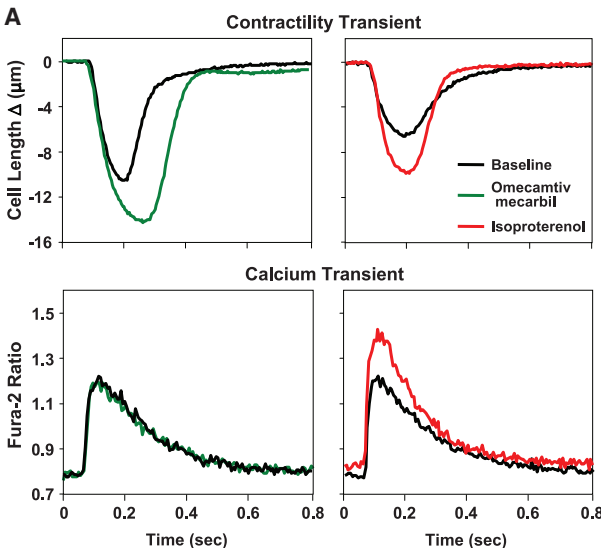


Fig. 3. The effects of omecamtiv mecarbil in cardiac myocytes. (A) Representative tracings showing that omecamtiv mecarbil (200 nM) increases cardiac myocyte contractility without changing the Ca^{2+} transient. In contrast, the β -adrenergic agonist isoproterenol (2 nM) increases contractility by increasing the Ca^{2+} transient. (B) Treatment

of myocytes ($n =$ eight myocytes per condition) with the β -adrenergic blocker, carvedilol, at a concentration (200 nM) sufficient to completely block the effect of isoproterenol (20 nM), does not alter the effect of omecamtiv mecarbil (200 nM) on myocyte contractility (FS). Data are means $\pm$ SEM.

measured by using coumarin-modified phosphate-binding protein as a fluorescent reporter of phosphate production (13). The rate of the subsequent step, ADP release, governs the length of time in the strongly bound state and did not change, nor did the rate of ATP binding and myosin release from the actin filament (fig. S4). These results explain the ability of omecamtiv mecarbil to increase the steady-state ATPase rate (fig. S5a) (14), because the weak-to-strong binding state transition is the rate-limiting step in the overall actin-myosin ATPase cycle.

To our surprise, when the rate of phosphate release was tested in the absence of actin, it was slowed by omecamtiv mecarbil, which decreased the overall ATPase rate of cardiac myosin in the absence of actin [median inhibitory concentration (IC_{50}) = 1.3 $\pm$ 0.09 μM (SD)] (Fig. 1E and figs. S5b and S6). In the presence of actin, the release of phosphate is intimately associated with the rate-limiting transition of myosin from a weakly actin-bound state to a strongly actin-bound force-producing state (12). Because omecamtiv mecarbil accelerated phosphate release only in the presence of actin, we postulated that omecamtiv mecarbil lowers the energy barrier for the transition from the weak to strong binding state in the actomyosin cycle. In a sarcomere containing an ensemble of myosin heads, of which the majority are not tightly bound to the actin filament at any moment during muscle contraction (11, 15), omecamtiv mecarbil is expected to increase the number of myosin heads interacting with actin filaments in a force-producing state. Because each head acts as an independent force generator, this results in an increased force output from the muscle.

To better understand the structural basis for the action of omecamtiv mecarbil on myosin, we used a benzophenone derivative of omecamtiv mecarbil (fig. S7) as a photo-activated affinity label to identify its potential binding site to cardiac

myosin S1. Tandem mass spectrometry consistently identified the same peptide (RSEAPPHF) (16) labeled by the omecamtiv mecarbil analog at serine 148 (table S1); competition with a high-affinity analog lacking the benzophenone labeling functionality eliminated this binding. Mapped onto the structure of the myosin S1 domain (17), the labeled peptide resides in a cleft large enough to accommodate omecamtiv mecarbil; its location is about 6.5 nm from the actin-binding interface and about 3 nm from the nucleotide-binding pocket (Fig. 2). The cleft is in a region where the relay helix and the converter domain converge at the base of the lever arm. These structural elements are thought to relay conformational changes in the nucleotide-binding pocket to produce motion in the lever arm that generates the power stroke. Consistent with this, crystallographic studies suggest that the position of the converter or lever arm can affect chemical transitions in the nucleotide-binding site (11, 12). Thus, the proposed site of omecamtiv mecarbil binding could allow it to allosterically modulate both the enzymatic and mechanical properties of the cardiac myosin motor.

On the basis of its site of action in the sarcomere and biochemical mechanism of action,

omecmtiv mecarbil should increase cardiac contractility without changes in cardiac myocyte calcium homeostasis. To test this hypothesis, we made simultaneous quantitative measurements of the contractile response and calcium transient in freshly isolated adult rat cardiac ventricular myocytes (18). Compared with control myocytes, omecamtiv mecarbil (200 nM) significantly increased the fractional shortening (percent cell length change) of adult rat cardiac myocytes (Table 1 and Fig. 3A, top left), but did not have any effect on the calcium transient as measured by the ratiometric fluorescent calcium indicator Fura-2 (Table 1 and Fig. 3A, bottom left). In contrast, the β -adrenergic agonist, isoproterenol, increased peak systolic and diastolic calcium and increased the rates of contraction and relaxation (Table 1 and Fig. 3A, right side). Omecamtiv mecarbil and isoproterenol were additive in terms of their effect on contractility, but addition of omecamtiv mecarbil to isoproterenol did not lead to any further changes in the calcium transient (Table 1). Higher concentrations of omecamtiv mecarbil (up to 10 μ M) did not increase the magnitude or kinetics of the calcium transient (table S2). As evident in the individual contractile transients shown (Fig. 3A), omecamtiv mecarbil

(200 nM) extended the duration of contraction, as indicated by an increase in the time to its peak ($156 \pm 7.4\%$, $P < 0.05$ compared with baseline) without affecting the rates of contraction or relaxation ($89 \pm 8.4\%$ and $101 \pm 11\%$, $P > 0.05$ compared with baseline) measured in the absence of Fura-2 (table S3). Omecamtiv mecarbil also increased myocyte contraction in the presence of carvedilol (Fig. 3B), a β -adrenergic blocker commonly used in heart failure patients (4). In summary, omecamtiv mecarbil increases the contractility of cardiac myocytes without affecting their calcium transient, in contrast to the β -adrenergic agonist isoproterenol.

Given the findings obtained in cardiac myocytes, we sought to understand how these effects of cardiac myosin activation would affect cardiac function in the intact animal. We used echocardiography to evaluate the effect of omecamtiv mecarbil infusions on the cardiac function of Sprague-Dawley rats and beagle dogs under isoflurane anesthesia. Measurements of systolic and diastolic left ventricular (LV) dimensions in the parasternal long axis (a cross-sectional view of the heart) were used to calculate an index of cardiac function, percent fractional shortening (FS). In both species, omecamtiv mecarbil produced statistically

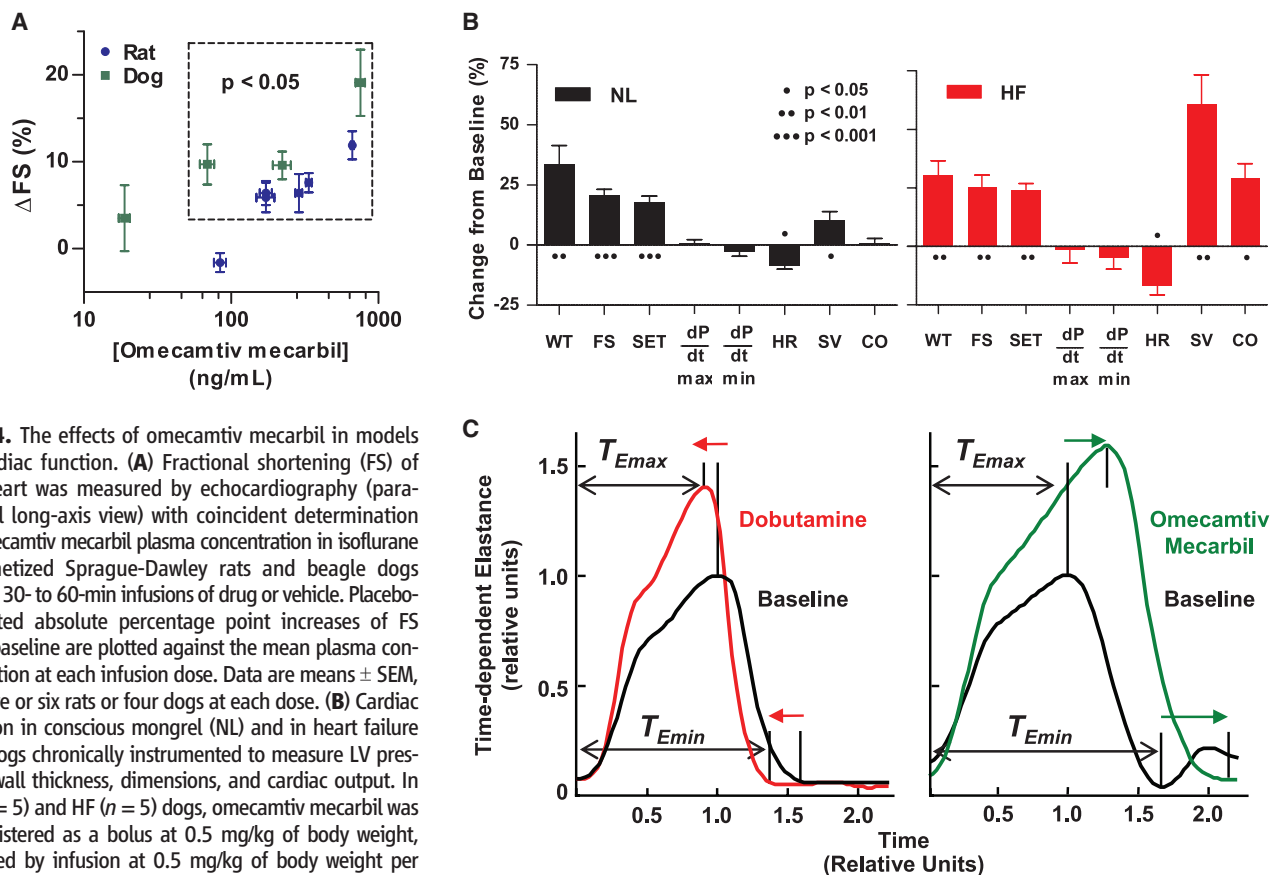


Fig. 4. The effects of omecamtiv mecarbil in models of cardiac function. **(A)** Fractional shortening (FS) of the heart was measured by echocardiography (parasternal long-axis view) with coincident determination of omecamtiv mecarbil plasma concentration in isoflurane anesthetized Sprague-Dawley rats and beagle dogs during 30- to 60-min infusions of drug or vehicle. Placebo-corrected absolute percentage point increases of FS from baseline are plotted against the mean plasma concentration at each infusion dose. Data are means $\pm$ SEM, $n =$ five or six rats or four dogs at each dose. **(B)** Cardiac function in conscious mongrel (NL) and in heart failure (HF) dogs chronically instrumented to measure LV pressure, wall thickness, dimensions, and cardiac output. In NL ($n = 5$) and HF ($n = 5$) dogs, omecamtiv mecarbil was administered as a bolus at 0.5 mg/kg of body weight, followed by infusion at 0.5 mg/kg of body weight per hour. Measurements plotted are mean $\pm$ SEM values 15 min after the start of drug administration. WT, wall thickening; FS, fractional shortening; SET, systolic ejection time; dP/dt, rate of pressure change; HR, heart rate; SV, stroke volume; CO, cardiac output. **(C)** Time-dependent elastance is plotted for dobutamine (10 μ g/kg per min) and omecamtiv

mecarbil (10 min following a 1 mg/kg bolus). The response of each dog is normalized to the magnitude and time of peak elastance at baseline to allow for comparison of the shape of the response. Statistics were performed using a one-way analysis of variance (ANOVA) with a post hoc Student-Newman-Keuls test.

significant, dose-dependent, concentration-dependent, and visually evident changes in cardiac contractility as indicated by increases in FS (Fig. 4A and movie S1) (19).

Omecamtiv mecarbil improved left ventricular systolic function in a conscious canine model with chronically implanted sensors to assess LV dimensions, atrial and arterial pressures, and stroke volume (20). The direct effect on cardiac contractility was evident from the increase in myocardial wall thickening (WT) and FS (Fig. 4B) in the absence of a change in loading conditions, such as mean arterial pressure, LV end diastolic pressure, and total vascular resistance ($-2.2 \pm 1.3\%$, $-7.3 \pm 5.8\%$, and $-2.4 \pm 2.3\%$, means $\pm$ SEM, $P > 0.05$). In dogs with heart failure induced by chronic fast pacing of heart rate in concert with a localized myocardial infarction (21), omecamtiv mecarbil produced substantially greater and statistically significant ($P < 0.01$) increases in stroke volume ($60.8 \pm 12.5\%$) and cardiac output ($29.1 \pm 6.1\%$) than it did in normal dogs ($10.2 \pm 3.6\%$ and $0.8 \pm 2.0\%$, respectively). The increase in cardiac output was especially notable given the coincident lowering of heart rate ($-16.7 \pm 4.0\%$, $P = 0.014$) observed in the dogs with heart failure (Fig. 4B; P calculated using Student's t test).

Underlying the effects on systolic function was an increase in systolic ejection time (SET) in the absence of changes in the rate of LV pressure development (dp/dt) (Fig. 4B). In contrast, existing drugs, such as the β -adrenergic agonist dobutamine, increase cardiac contractility by increasing dp/dt and shortening SET (22). We investigated this finding further by comparing omecamtiv mecarbil with dobutamine, using time-dependent LV end systolic elastance, a load-independent measure of cardiac contractility derived from the pressure-volume loop (23). The plots of time-dependent elastance (Fig. 4C) are illustrative of the different effects that the two drug mechanisms have on the dynamics of cardiac contractility.

Overall energy balance in the contracting heart is set by a combination of loading conditions, heart rate, membrane ion fluxes, calcium cycling, and crossbridge cycling. Although omecamtiv mecarbil might increase ATP turnover at the level of the sarcomere, on balance, myocardial energetics appear unchanged following omecamtiv mecarbil administration, as it does not increase overall myocardial oxygen consumption (8) at doses producing substantial improvements in cardiac function. However, excessive crossbridge activation at excessive doses of omecamtiv mecarbil could lead to an increase in the duration of systole to an extent where coronary blood flow during diastole is reduced, and signs and symptoms of cardiac ischemia may emerge.

As a selective, allosteric activator of cardiac myosin, omecamtiv mecarbil is a rare example of a drug mechanism whose action depends on activation rather than inhibition of an enzyme, an approach that may have broader application

for therapeutic intervention (24–26). It represents a therapeutic approach to directly improve cardiac function that potentially avoids the deleterious effects limiting current indirect inotropic mechanisms (27). Further studies in patients with heart failure will eventually define the clinical benefit and risk profile of cardiac myosin activation in a condition that is still marked by substantial rates of mortality and morbidity.

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- Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
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- In the online materials, a side-by-side movie shows several heartbeats of a short-axis echocardiogram in an anesthetized beagle before (on the left) and after (on the right) a 1-hour infusion of omecamtiv mecarbil at 1 mg/kg of body weight per hour. In this view, the left ventricular cavity is in the middle of a ring of contracting myocardium.
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Supporting Online Material

www.sciencemag.org/cgi/content/full/331/6023/1439/DC1
Materials and Methods

Figs. S1 to S9

Tables S1 to S3

References

Movie S1

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Reversal of Interlaminar Signal Between Sensory and Memory Processing in Monkey Temporal Cortex

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The primate temporal cortex implements visual long-term memory. However, how its interlaminar circuitry executes cognitive computations is poorly understood. Using linear-array multicontact electrodes, we simultaneously recorded unit activities across cortical layers in the perirhinal cortex of macaques performing a pair-association memory task. Cortical layers were estimated on the basis of current source density profiles with histological verifications, and the interlaminar signal flow was determined with cross-correlation analysis between spike trains. During the cue period, canonical “feed-forward” signals flowed from granular to supragranular layers and from supragranular to infragranular layers. During the delay period, however, the signal flow reversed to the “feed-back” direction: from infragranular to supragranular layers. This reversal of signal flow highlights how the temporal cortex differentially recruits its laminar circuits for sensory and mnemonic processing.

The primate inferotemporal cortex locates at the final stage of the ventral visual pathway and serves as a storehouse for visual long-

term memory (1–4). Previous studies have demonstrated neuronal activity related to presented visual objects and retrieved images at the single-neuron level (4–6), but the underlying network dynamics (7–12) remain to be understood. Evidence from the primary sensory cortices suggests that local circuits extending across cortical layers are crucially involved in sensory processing (13–15).

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This raises questions about how the interlaminar circuitry in the inferotemporal cortex is differentially recruited to process presented objects and to retrieve visual long-term memory.

We used two strategies to investigate interlaminar signal flow in awake behaving monkeys. First, we used current source density (CSD) analysis as a tool for layer estimation in each electrode penetration; CSD reflects the gross transmembrane currents in the local neuronal ensemble and is used to estimate the cortical layers that receive afferent inputs (16, 17). Second, we used cross-correlation analysis of spike trains (18–21) to infer the functional interactions across cortical layers; asymmetry or peak lag of the cross-correlogram (CCG) reflects the direction of functional connectivity between neurons (22, 23).

Two monkeys were trained to perform a pair-association task, in which they had to retrieve the learned paired associate in response to the presented cue stimulus (Fig. 1A) (3–5). We recorded single- and multi-unit activities and local field potentials (LFPs) by inserting linear-array multicontact electrodes (16 or 24 contacts with spacing of 150 or 100 μm , respectively) vertically (table S1) (24) into area 36 (A36) (Fig. 1B). CSD was then calculated from depth profiles of stimulus-evoked LFPs in order to physiologically estimate the position of the granular layer (24). A representative CSD profile exhibited the earliest current sink (Fig. 1, D and E, asterisks) at the contact corresponding to the histologically verified granular layer (Fig. 1C, red). This earliest current sink was followed by sinks at more superficial contacts and by sources at deeper contacts (Fig. 1D). Similar CSD profiles were consistently observed for all penetrations (fig. S1). Postmortem histological analyses (24) confirmed that the earliest current sink evoked by cue stimuli consistently corresponded to the granular layer [table S1, the distance between the contact with the earliest current sink (“earliest-sink contact”), and the center of the granular layer was 79 μm (median), $n = 6$ penetrations]. The histological verifications, together with consistent CSD profiles across penetrations, demonstrated that the CSD profiles can be reliably used to estimate the granular layer (G), the supragranular layer (SG), and the infragranular layer (IG) (24). In this representative penetration, single unit activities were simultaneously recorded in SG and IG (Fig. 1, F and G), both showing stimulus-selective responses during the cue period (Fig. 1H). The CCG (18–23) for this unit pair exhibited a significant displaced peak (4 ms lag) on the right side (Fig. 1I), suggesting a functional connectivity from the SG unit to the IG unit, which is consistent with the “feed-forward” signal flow in the primary sensory cortices (13–15).

We made 20 penetrations in three hemispheres of two monkeys and conducted cross-correlation analyses for three populations of unit pairs: G-SG pairs (cue period, $n = 52$ pairs; delay-period, $n = 49$ pairs), G-IG pairs ($n = 128$ pairs; $n = 121$ pairs), and SG-IG pairs ($n = 252$ pairs; $n = 211$ pairs) [both single units and multi-units were

included; for details, see supporting online material (SOM) text and table S2]. A CCG was calculated only when both constituent units responded to at least one common stimulus during either the cue or delay period. CCG peak was detected within 10 ms lag (19–22) so as to evaluate its significance ($Z > 2.82$, $P < 0.05$) (24). We then compared the proportions of unit pairs with significant CCG peaks among the G-SG, G-IG, and SG-IG pairs

(fig. S2). The proportion of unit pairs with a significant CCG peak was greater for G-SG pairs than for G-IG pairs during both the cue period (33% versus 11%; χ^2 test with post-hoc pair-wise comparisons followed by Bonferroni’s correction, $P < 0.005$) and delay period (27% versus 12%; $P < 0.05$). The proportion of unit pairs with a significant CCG peak was greater for G-SG pairs than for SG-IG pairs during the cue period (33% versus 16%;

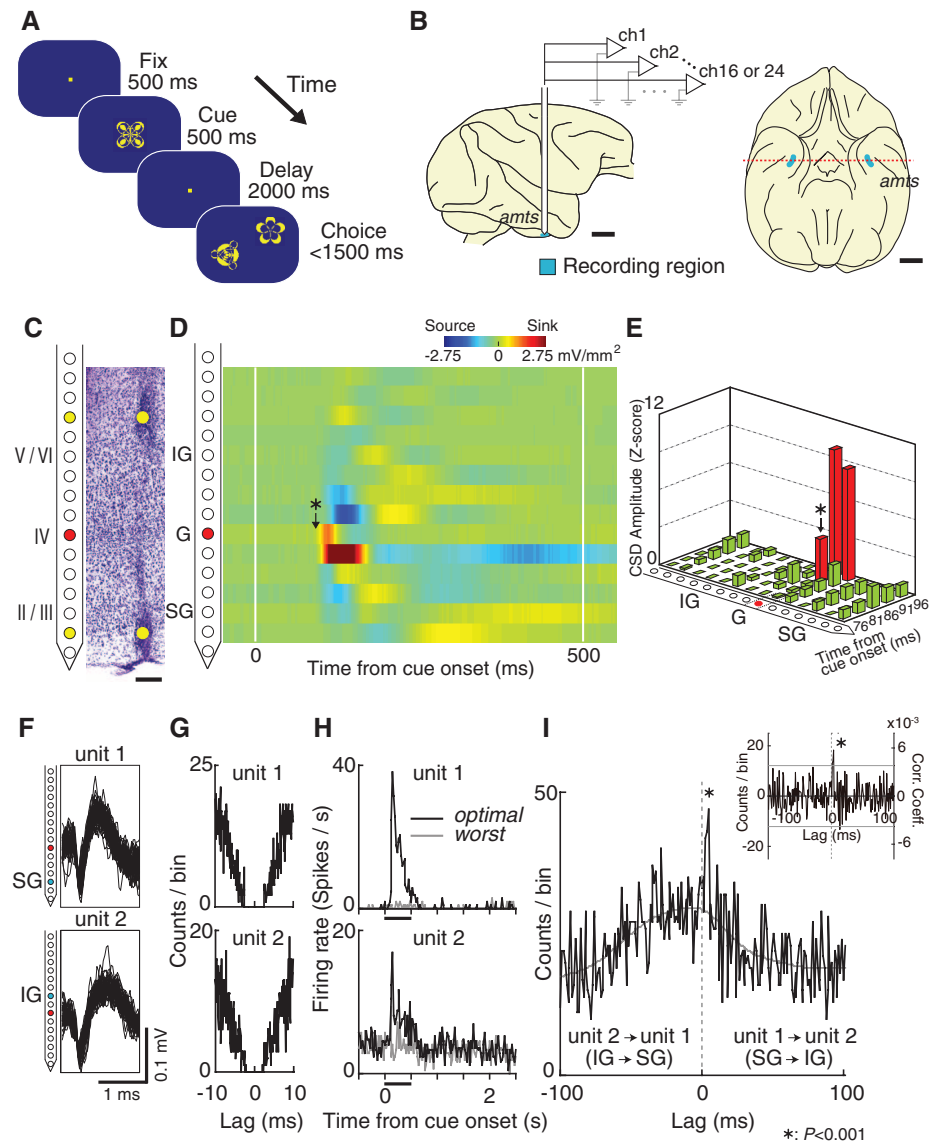


Fig. 1. (A) Sequence of pair-association task. Monkeys had to retrieve the learned paired associate in response to the presented cue stimulus. (B) Lateral (left) and ventral (right) views of monkey brain. Unit activities and LFPs were recorded across cortical layers in area 36 (blue) by using a linear-array multicontact electrode. Scale bar, 10 mm; amts, anterior middle temporal sulcus. (C to I) A representative data set. (C) Electrolytic lesion marks made at two contacts of the electrode (yellow contacts) were identified in a Nissl-stained histological section. Scale bar, 200 μm . [(D) and (E)] Stimulus-evoked CSDs. The earliest significant current sink appeared at 91 ms after cue onset (asterisks), (red contact). The red contact corresponded to the granular layer in histological section (C). Red and green bars in (E) indicate significant and nonsignificant current sink, respectively. G, SG, and IG represent granular, supragranular, and infragranular layers, respectively. (F) Waveforms, (G) auto-correlograms, and (H) poststimulus time histograms of two single units simultaneously recorded in SG and IG [(F), blue contacts]. (I) Raw CCG between spike trains of the SG and IG units in the cue period (black line). Gray line, IFR-predictor (24). Bin width, 1 ms. (Inset) IFR-predictor-subtracted CCG. The CCG exhibited a significant peak on the right side (lag time, 4 ms). Horizontal gray line indicates the confidence limit.

$P < 0.05$), and the same tendency was observed during the delay period (27% versus 19%; $P = 0.24$).

We next examined the direction of functional connectivity between units in different layers during each task period. For G-SG pairs, the distribution of asymmetry index (AI) (22–24) of individual CCGs during the cue period was shifted to the feed-forward direction: from G to SG (Fig. 2B, blue) [Wilcoxon signed-rank test; cue period (Fig. 2B, blue), $P < 0.01$, $n = 17$ pairs]. This directional bias was not significant during the delay period (Fig. 2B, red) ($P > 0.4$, $n = 14$ pairs). Similar results were obtained using the center of mass (CoM) of the CCG peak (Fig. 2C) [cue period (Fig. 2C, blue), $P < 0.04$; delay period (Fig. 2C,

red), $P > 0.5$]. During the fix period, only four pairs exhibited a significant CCG peak, and thus the directional bias was not statistically evaluated. These results were further substantiated by the population-averaged CCGs (Fig. 2A and fig. S4A): The CCG showed a prominent peak [$P < 0.001$, (24)] on the right side during the cue period (G to SG) (Fig. 2A, blue). In G-IG pairs, no bias was observed in their signal flow directions during any of the task periods (fig. S3).

We repeated the same analyses for SG-IG pairs (Fig. 2, E to G). The distribution of AIs during the cue period was significantly shifted toward the direction from SG to IG (Fig. 2F, blue) ($P < 0.01$, $n = 41$ pairs). However, the distribution

during the delay period exhibited a bias in the opposite direction, IG toward SG (Fig. 2F, red) ($P < 0.01$, $n = 41$ pairs). No significant directional bias was observed during the fix period ($P > 0.2$, $n = 12$ pairs). Similar results were obtained using the CoM [cue period (Fig. 2G, blue), $P < 0.03$; delay-period (Fig. 2G, red), $P < 0.02$; fix-period, $P > 0.8$] (24). Population-averaged CCGs again confirmed these results (Fig. 2E and fig. S4B): During the cue period, a significant peak was observed on the right side ($P < 0.001$; SG to IG, Fig. 2E, blue), whereas a significant peak appeared on the left side during the delay period ($P < 0.001$; IG to SG, Fig. 2E, red). Consistent results were obtained using only single unit data (SOM text and fig. S5).

These results demonstrated the signal flow from G to SG and from SG to IG during the cue period, as in the canonical feed-forward processing (Fig. 2, D and H, left) (13–15). During the delay period, however, the direction of signal flow reversed, suggesting recruitment of a “feed-back” pathway (Fig. 2H, right).

We then examined the temporal dynamics of the functional connectivity for individual SG-IG pairs. Figure 3A shows the time course of the correlation strength (CS) and asymmetry index (AI) for each pair ($n = 70$ pairs) that exhibited a significant peak during either the cue or delay period (24). Although the connectivity of individual pairs exhibited a variety of dynamics, as a whole the direction of connectivity gradually changed: SG to IG (Fig. 3A, blue) during the cue period and IG to SG (Fig. 3A, orange) during the delay period (Fig. 3B) (for the temporal dynamics of firing rates, see SOM text and figs. S6 and S7). To investigate these observations quantitatively, we divided SG-IG pairs according to the sign of the AI in the cue and delay periods. Nearly half of the pairs (47%, 33 of 70) exhibited directional changes in the connectivity between the cue and delay periods (“flipped pairs”) (Fig. 3C), suggesting that the signal flow direction can be modulated in individual pairs. Of these, a significantly greater proportion (73%, 24 of 33 pairs; χ^2 test, $P < 0.01$) exhibited connectivity from SG to IG during the cue period and reversed their direction during the delay period. Furthermore, unit pairs that did not change the sign of AI between the cue and delay periods (“non-flipped pairs”; 53%, 37 of 70 pairs) also contributed to the overall changes in the signal flow (Fig. 3D): For unit pairs with connectivity direction from SG to IG (Fig. 3D, blue), AIs in the delay period were closer to zero than those in the cue period (Wilcoxon signed-rank test, $P < 0.05$, $n = 17$ pairs), and for unit pairs with connectivity direction from IG to SG (Fig. 3D, orange), AIs in the delay period were more negative than those in the cue period ($P < 0.01$, $n = 20$ pairs). Together, the reversal of connectivity direction between the cue and delay periods (Fig. 2) was the result of both the directional changes of the flipped pairs and consistent small directional shifts of the non-flipped pairs.

Lastly, we examined the spatial patterns of functional connectivity by calculating the laminar

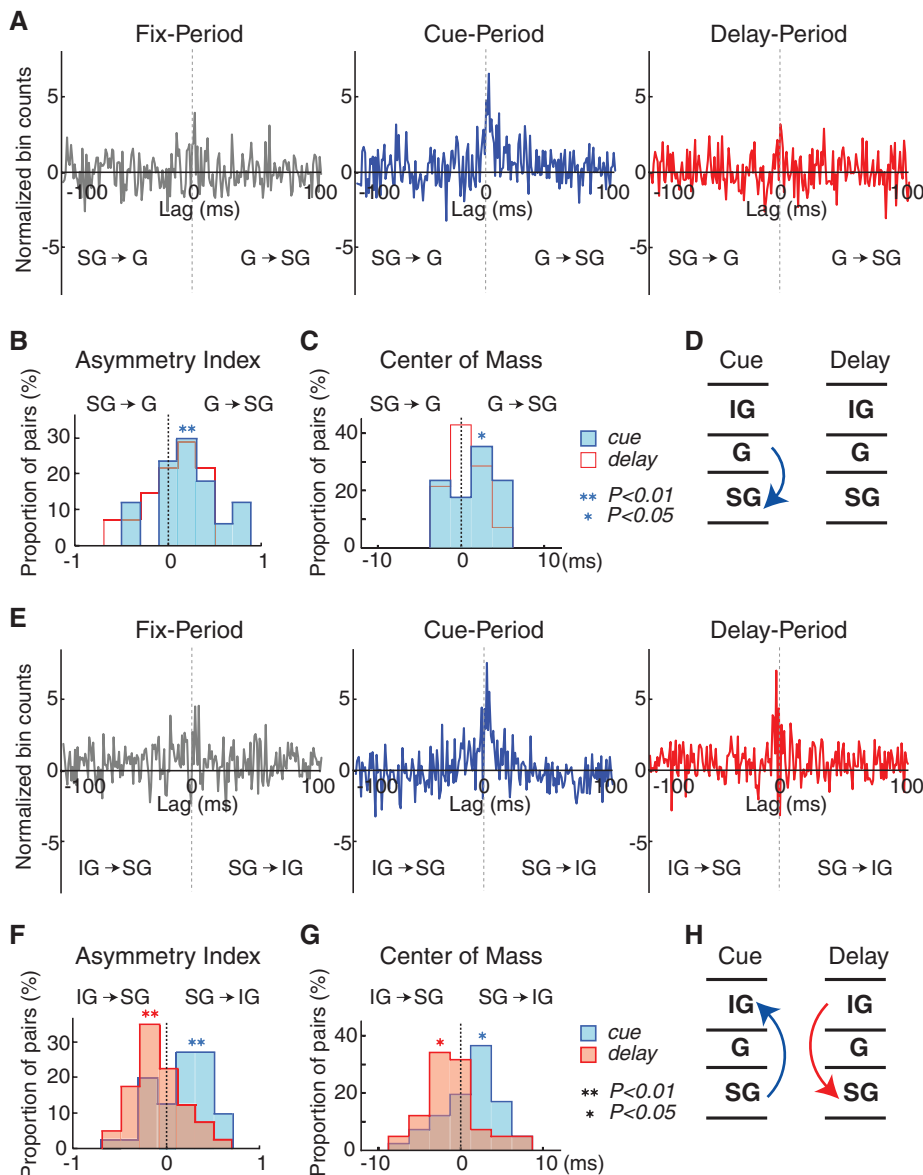


Fig. 2. Population results of the functional connectivity. (A to C) Cross-correlation between spike trains of G-SG pairs. (A) Population-averaged CCGs in the (gray, left) fix, (blue, middle) cue, and (red, right) delay periods, respectively. (B) Asymmetry index and (C) center of mass of individual CCGs. Asterisk indicates significant bias to either side of the histogram. Filled histogram indicates a task period for which significant bias in the directionality was observed. (D) Schematic diagram of interlaminar signal flow between G and SG. (E to H) Same as in (A) to (D), but for SG-IG pairs.

positions of units by parametrically using the distances from the estimated granular layer (Fig. 4 and fig. S8) (25, 26). Compared with the connectivity during the fix period, two prominent connectivity patterns appeared during the cue period, corre-

sponding to the feed-forward pathways from G to SG and from SG to IG (Fig. 4, A and B, middle). During the delay period, the feed-forward connectivity was attenuated, and the feed-back connectivity from IG to SG emerged (Fig. 4, A and B, right). In

addition, outward signal flow (from superficial to deep parts) within IG was found during the delay period (Fig. 4, A and B, right). Putative target units of this outward flow were located at significantly deeper positions than those of the putative source

Fig. 3. Connectivity dynamics of individual SG-IG pairs. **(A)** Time course of spike correlation for individual unit pairs. AI and CS of CCGs were color-coded as shown in the inset. Unit pairs were sorted according to AI value during the latter half of the delay period. **(B)** Population average of all the unit pairs. **(C and D)** Polar plot of CS and AI dynamics for the (C) flipped- and (D) non-flipped pairs. Radius, CS. Angle from the vertical axis, AI. Positions of base and tip of an arrow correspond to AI/CS values during cue and delay periods, respectively. [(C, right)] Proportion of each type of flipped pairs. S and I represent SG and IG, respectively. [(D, right)] AI of non-flipped pairs during the cue and delay periods. Blue, SG→IG pairs; orange, IG→SG pairs.

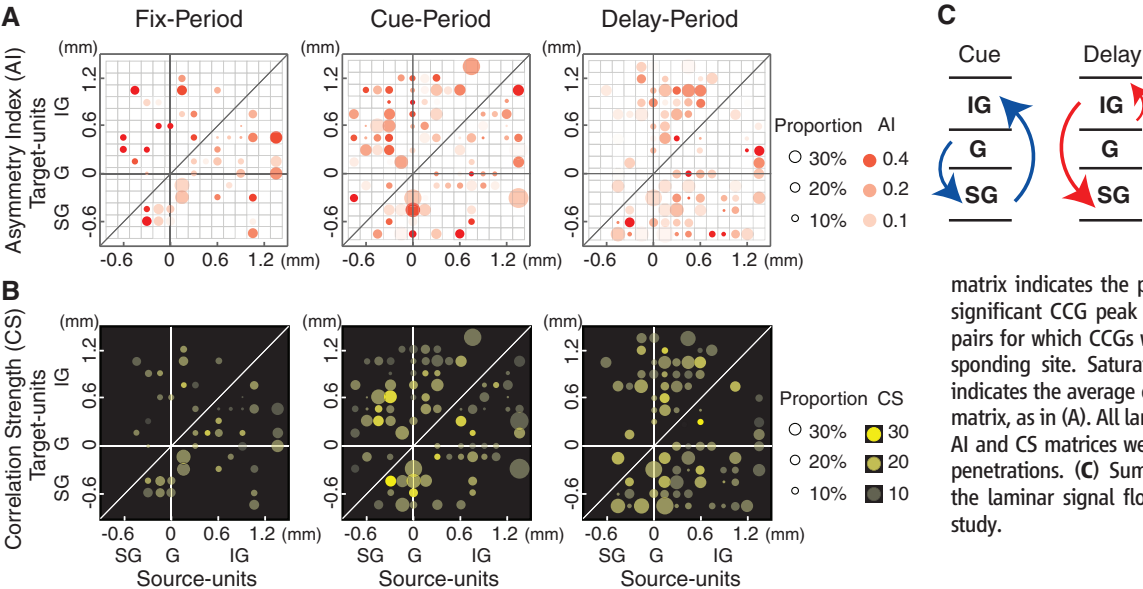
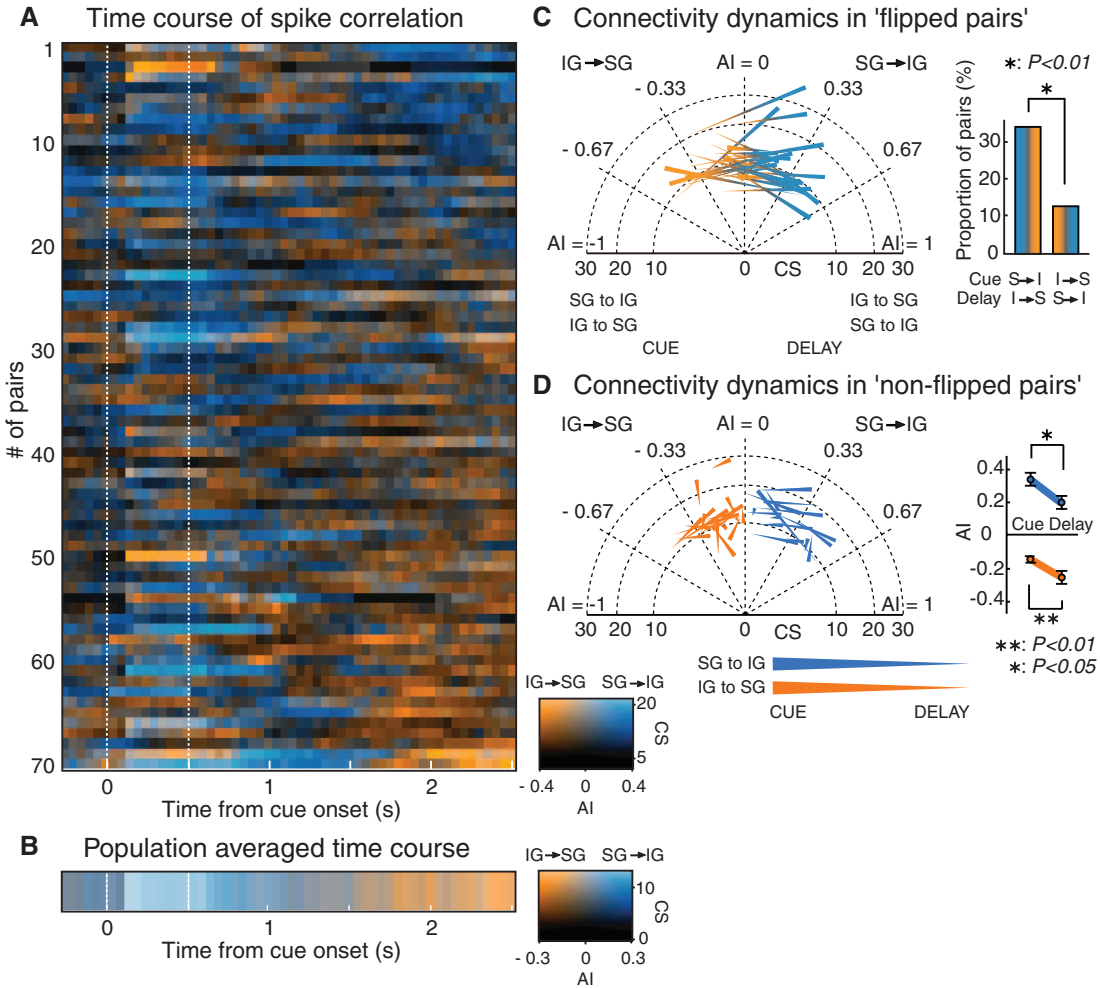


Fig. 4. Interlaminar connectivity matrices. **(A)** AI matrix for each task period. Abscissa and ordinate represent recorded positions of the putative source and target units relative to the earliest-sink contact, respectively. Size of a circle in each matrix indicates the proportion of unit pairs with significant CCG peak to the total number of unit pairs for which CCGs were calculated at the corresponding site. Saturation of color of each circle indicates the average of AI across unit pairs. **(B)** CS matrix, as in (A). All laminar positions plotted in the AI and CS matrices were recorded in at least three penetrations. **(C)** Summary diagrams showing all the laminar signal flows identified in the present study.

units (fig. S9) [paired *t* test, $P < 0.02$, $n = 19$ pairs; median distances from the granular layer were 0.45 mm (source units) and 1.05 mm (target units)].

The present study demonstrated that canonical feed-forward signal flow across cortical layers during sensory coding reverse to the feed-back direction during memory retrieval phase, which suggests flexible recruitment of interlaminar connectivity depending on the cognitive demands in the monkey association cortices (Fig. 4C). We used CSD analysis to estimate cortical layers (Fig. 1, C to E, and fig. S1), and the observed stimulus-evoked CSD profiles were quite similar to those in the primary sensory cortices (17, 27). For some penetrations, we observed that the current sink positioned superficially next to the earliest-sink contact exhibited larger peak amplitudes and much longer durations than that of the earliest current sink. This observation might reflect the cytoarchitectural nature of A36 as a dysgranular cortex (28) as well as the direct inputs to the deepest part of the superficial layer, which is consistent with anatomical observations (29).

A recent study in the rat primary auditory cortex demonstrated that the direction of interlaminar signal flow depends on the cortical “state”: Sensory-evoked responses were initiated in the thalamorecipient layers and then propagated to the superficial and deep layers, whereas in spontaneously active “up-states,” neuronal activity was initiated in the deep layers and then propagated to the superficial layers (27). These state-dependent

changes in the interlaminar signal flows in rats are consistent with our results obtained in monkeys performing a memory task. Together, these findings highlight the flexibility of cortical laminar circuits. Further experiments will be needed to determine whether such flexible interlaminar connectivity is also implemented and used in other cortical areas for other cognitive demands.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/331/6023/1443/DC1
Materials and Methods
SOM Text
Figs. S1 to S9
Tables S1 and S2
References

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A Brief Social-Belonging Intervention Improves Academic and Health Outcomes of Minority Students

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A brief intervention aimed at buttressing college freshmen's sense of social belonging in school was tested in a randomized controlled trial ($N = 92$), and its academic and health-related consequences over 3 years are reported. The intervention aimed to lessen psychological perceptions of threat on campus by framing social adversity as common and transient. It used subtle attitude-change strategies to lead participants to self-generate the intervention message. The intervention was expected to be particularly beneficial to African-American students ($N = 49$), a stereotyped and socially marginalized group in academics, and less so to European-American students ($N = 43$). Consistent with these expectations, over the 3-year observation period the intervention raised African Americans' grade-point average (GPA) relative to multiple control groups and halved the minority achievement gap. This performance boost was mediated by the effect of the intervention on subjective construal: It prevented students from seeing adversity on campus as an indictment of their belonging. Additionally, the intervention improved African Americans' self-reported health and well-being and reduced their reported number of doctor visits 3 years postintervention. Senior-year surveys indicated no awareness among participants of the intervention's impact. The results suggest that social belonging is a psychological lever where targeted intervention can have broad consequences that lessen inequalities in achievement and health.

An important question facing society concerns the origins of inequalities between socially marginalized and nonmarginalized groups. Among the most consequential of inequalities is the poorer school and health outcomes experienced by African Americans, Latino

Americans, and other non-Asian ethnic minorities relative to European Americans. These differences occur at all levels of socioeconomic status (1–3).

Although many structural factors contribute to these inequalities, the present research exam-

ines a psychological factor: concern about social belonging. Social belonging—a sense of having positive relationships with others—is a fundamental human need (4, 5). Social isolation, loneliness, and low social status harm not only subjective well-being (6) but also intellectual achievement (7) and immune function and health (8–11). Even a single instance of exclusion can undermine well-being (12, 13), intelligence quotient (IQ) test performance, and self-control (14).

Members of socially stigmatized groups, such as African Americans, may be relatively more uncertain about their social belonging in mainstream institutions like school and work (7). Because their ethnic group is often negatively stereotyped and marginalized, they may be unsure of whether they will be fully included in positive social relationships in these settings (2). As the sociologist Erving Goffman wrote, “The central feature of the stigmatized individual's situation in life...is a question of...‘acceptance’” (15). Uncertainty about belonging, especially when chronic, can undermine minorities' performance (7, 16) and health (3, 17, 18). Social belonging may thus constitute a psychological lever where targeted intervention could yield broad benefits.

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Such an intervention is reported here. Critical to its rationale is the insight that it is people's subjective interpretations of the quality of their relationships, more so than the objective number or attributes of those relationships, that strongly affects well-being (5, 19). The present intervention, delivered to students during the challenging transition to college, was designed to encourage nonthreatening interpretations of adversity. During the transition to a new school, students can face frequent social setbacks and feelings of isolation. Their well-being and performance may depend, to a great extent, on whether they construe such experiences as evidence that they do not belong.

Because African-American students experience relatively greater uncertainty about their belonging in school, they were expected to benefit from the intervention more than nonminority students (7). Further if, as we intended, the intervention triggered an enduring perceptual change in the encoding of social experience, its effects might persist over time. Short-term effects might compound into long-term effects through a recursive virtuous cycle, in which early performance gains assure students of their belonging in school, which in turn improves their performance, in a repeating feedback loop (20). Students who feel more assured of their belonging may also initiate more social interactions and form better relationships on campus, facilitating their social integration and further benefiting their well-being, performance, and health (21).

The intervention was delivered to two cohorts of African-American ($N = 49$) and European-American ($N = 43$) students in the second semester of their first year at a selective college (22, 23). To assess psychological responses to adversity, we asked participants to complete daily surveys in the first week after the intervention. To assess their long-term sense of belonging, health, and well-being, we asked them to complete an end-of-college survey 3 years later (completion rate 78.26%) (23). At the end of this survey, participants were asked to authorize the release of their complete college academic transcript (authorization rate 97.22%) (23).

Participating students were randomly assigned to either the belonging-treatment condition or a control condition. In cohort 1, participants were recruited through convenience sampling; in cohort 2, through random sampling (23). An additional campus-wide control group was obtained by collecting the anonymized official grade-point averages (GPAs) of all European Americans ($N = 1362$) and African Americans ($N = 194$) in the same class years as participants but who did not participate in the study (23). This group was included in secondary analyses of GPA.

The intervention provided students with a narrative that framed social adversity in school as shared and short-lived (24). This message encouraged students to attribute adversity not to fixed deficits unique to themselves or their ethnic group but to common and transient aspects of the

college-adjustment process. Upon arrival in a research lab, participants read a report of the ostensible results of a survey of more senior students at their school. Most students, the report indicated, had worried about whether they belonged in college during the difficult first year but grew confident in their belonging with time. The survey results were said to be consistent across ethnic and gender groups. For instance, one survey respondent was quoted as saying, "Freshman year even though I met large numbers of people, I didn't have a small group of close friends...I was pretty homesick, and I had to remind myself that making close friends takes time. Since then...I have met people some of whom are now just as close as my friends in high school were" (23). Concerns about belonging were thus represented as common at first, as temporary, and as due to the challenging nature of the college transition.

To encourage participants to internalize the message, several steps exploited the "saying-is-believing effect"—the tendency to endorse messages that one has freely advocated (25). Participants were asked to write an essay describing how their own experiences in college echoed the experiences summarized in the survey. They then turned their essay into a speech, which they delivered to a video camera. These materials, participants were told, would be shown to future students to help ease their transition to college. Beyond facilitating internalization, this procedure averted the potential stigma of receiving an intervention, because it encouraged participants to see themselves as benefactors and not as beneficiaries (24, 26). In the control condition, the procedure was the same but the survey addressed topics unrelated to belonging (e.g., change in social-political attitudes) (23). The intervention lasted about 1 hour.

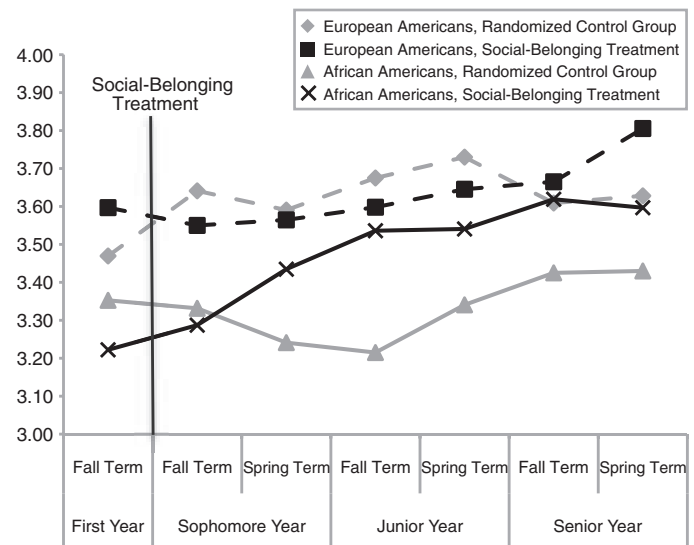
Few analyses were moderated by cohort (i.e., no more than would be expected by chance alone and none involving the primary outcomes of GPA, health, or well-being). Thus, data from the two cohorts were combined to increase statistical

power. First, analyses examined the trajectory of students' official GPA over time. In contrast to all other groups, African Americans in the control group showed no improvement in GPA from the fall of their freshman year, the semester before the intervention, through their sophomore, junior, and senior years [linear trend $F < 1$]. By contrast, the GPAs of intervention-treated African Americans rose over time [for linear trend, $F(1,34) = 13.79$, $P = 0.0007$; for time $\times$ condition, $F(1,34) = 4.16$, $P = 0.049$]. The GPAs of European-American students also rose over time [$F(1,29) = 6.88$, $P = 0.014$] with no difference by condition [$F < 1$]. As Fig. 1 shows, the intervention set African Americans on an upward trajectory such that the gap between them and their European Americans classmates closed over time. By students' senior year, the gap was cut by 79% (23).

Multiple regression, with student gender controlled, tested the effect of student race and condition (randomized control versus social-belonging treatment) on change in GPA—mean postintervention GPA (sophomore through senior years) minus mean preintervention GPA (fall term, first year) (23). There was no condition effect on preintervention GPA for either racial group [t values < 1] (table S1) (23). However, a significant condition effect on change in GPA emerged for African Americans [$B = 0.30$, $t(65) = 2.54$, $P = 0.014$] with no effect for European Americans [$t < 1$] [race $\times$ condition $B = -0.31$, $t(64) = -2.27$, $P = 0.027$]. The intervention closed the minority gap in 3-year GPA (SD = 0.36) from 0.29 points in the control condition to 0.14 points in the treatment condition, a 52% reduction.

Adding the campus-wide sample further supports treatment efficacy. An agreement with uni-

Fig. 1. Raw GPA by student race, experimental condition, and academic term. Means are noncumulative and were combined across cohorts. Ranges in sample sizes and standard errors for European Americans are $N = 25$ to 33 and $SE = 0.08$ to 0.14; for African Americans, $N = 30$ to 37 and $SE = 0.09$ to 0.12.



versity officials precludes the reporting of raw or adjusted means in this sample. To honor this agreement but present the results graphically, we performed analyses on residual postintervention GPA with preintervention GPA and gender controlled. Multiple regression on change in GPA and on raw postintervention GPA with preintervention GPA included as a covariate yield virtually identical results (23). As shown in Fig. 2A, treated African Americans had higher residual GPA scores than did African Americans campus-wide [$B = 0.28$, $t(1620) = 3.97$, $P = 0.00008$] and African Americans in the randomized control group [$B = 0.24$, $t(1620) = 2.62$, $P = 0.009$]. The latter two groups did not differ [$t < 1$]. (Fig. 2A) (23).

Illustrating its broad impact, the intervention tripled the percentage of African Americans earning postintervention GPAs in the top 25% of their class, as measured by both residual and raw postintervention GPA, and tended to reduce the percentage of African Americans performing in the bottom 25% of their class on both indices (Fig. 2, B and C) (23).

What accounts for these treatment effects? Daily surveys, collected the week after the intervention, suggest that the intervention buffered African Americans against adversity (23). Among untreated African Americans, feelings of belonging in school rose and fell with the degree of adversity students reported having experienced earlier that day and the day before. As adversity rose, belonging fell (mean within-subjects $R = -0.45$, derived from the average of individual participants' within-subjects correlations, after each was subjected to a Fisher r -to- z transformation) (23). For treated African Americans, this relationship was reduced to nil [mean within-subjects $R = 0.01$], a significant reduction [$t(59) =$

2.99 , $P = 0.004$]. In summary, the intervention robbed adversity of its symbolic meaning for African Americans, untethering their sense of belonging from daily hardship (27). Like treated African Americans, European Americans showed little relationship between adversity and belonging [for both conditions, mean within-subjects $R = -0.09$; condition difference, $t < 1$] [race $\times$ condition: $t(59) = -2.04$, $P = 0.046$].

These results provide a window into the shift in African-American students' psychology caused by the intervention. This shift benefited their long-term performance. African Americans whose belonging was more robust to daily adversity—whose sense of belonging was relatively independent of their day-to-day adversity—showed greater improvement in their 3-year postintervention GPA [$R = 0.51$, $P = 0.001$] (23). The effect of the intervention in protecting African-American students' sense of belonging from daily adversity mediated its effect on their GPA (23). The intervention thus planted a change in social perception that, it appears, accompanied students long after the intervention ended to affect their performance in college.

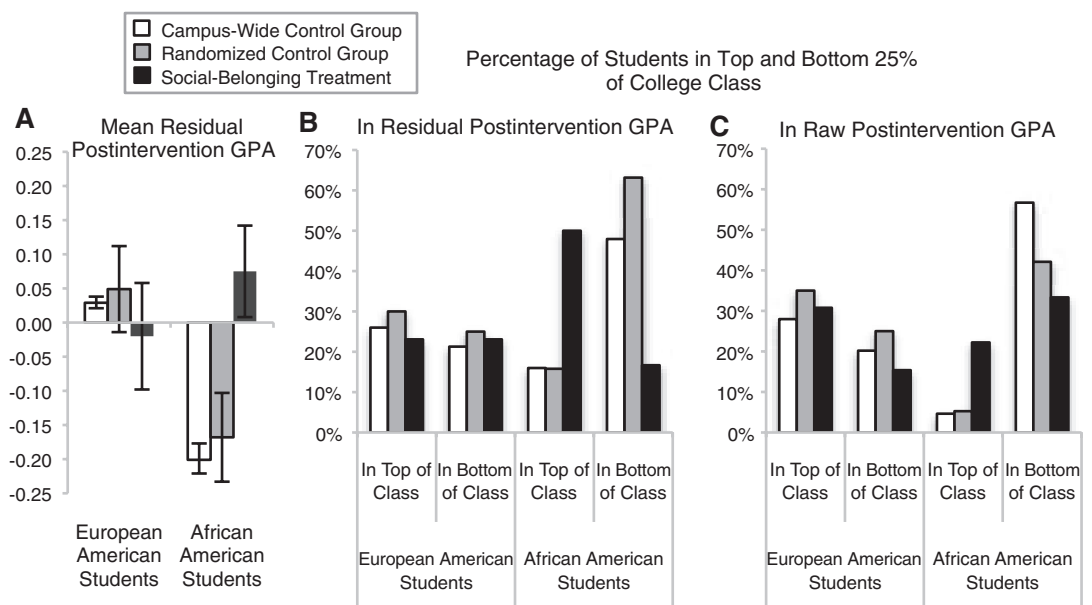
Three years after the intervention, at the end of their college tenure, participants completed a survey to assess long-term effects on psychology, well-being, and health. Also, to assess whether the intervention operated beneath conscious awareness, we asked participants whether they remembered the intervention from 3 years previously, whether they thought it had affected them, and whether they agreed with its message. On no outcome did European Americans differ by condition [t values < 1.35 , P values > 0.18]. African Americans, however, showed consistent treatment effects. The race $\times$ condition interaction

was not always significant, indicating that the treatment effect was not always larger for African Americans than for European Americans. Degrees of freedom vary because some measures were completed only by participants in cohort 2 (23).

If the intervention lessened how much African Americans' belonging fluctuated with adversity, and if it did so by lessening how much they viewed campus life through the lens of race, then intervention-treated African Americans should (i) report greater stability and less uncertainty about their belonging in school [less agreement with items like, "When something bad happens, I feel that maybe I don't belong at [school name]"] (7) and (ii) exhibit less cognitive accessibility of negative racial stereotypes and self-doubt (23). They did [self-reported belonging uncertainty, $t(36) = -2.01$, $P = 0.052$; accessibility of negative racial stereotypes, $t(66) = -2.01$, $P = 0.049$; accessibility of self-doubt, $t(64) = -2.64$, $P = 0.010$] (Fig. 3) (23).

Given the importance of social belonging for reducing stress and improving immune function and physical health (5, 8–11, 19) and the relatively poorer health experienced by African Americans, even those high in socioeconomic status (3), we examined effects on health. We assessed self-reported health, an important predictor of morbidity and mortality (28), using the five-item general health component of the Medical Outcomes Study Short-Form Health Survey (23). We also asked participants how many times they had visited the doctor in the previous 3 months (cohort 1) or 1 month (cohort 2). African Americans reported being healthier and visiting the doctor less frequently in the treatment condition than in the control condition [$t(32) = 2.48$, $P = 0.019$ and $t(63) = -2.23$, $P = 0.030$,

Fig. 2. Cumulative academic performance from sophomore through senior year. Data were combined across cohorts. (A) Residual sophomore-through-senior-year GPAs adjusted for student gender and preintervention (fall term, first year) GPA. Error bars represent ± 1 SE. Means represent the degree to which students performed better (positive values) or worse (negative values) than expected after the intervention in GPA units based on their gender and preintervention performance. (B) Percentage of students in the top and bottom 25% of their college class in residual postintervention GPA (i.e., postintervention GPA adjusted for student gender and preintervention GPA). (C) Percentage of students in the top and bottom 25% of their college class in raw postintervention GPA. For analytic details, see (23). Sample sizes for European Americans are $N_{\text{campus-wide control group}} = 1362$ and $N_{\text{experimental groups}} = 33$; for African Americans, $N_{\text{campus-wide control group}} = 194$ and $N_{\text{experimental groups}} = 37$.



respectively] (Fig. 4, A and B). Whereas 60% of untreated African Americans had seen a doctor recently, only 28% of treated African Americans had [$\chi^2(1, N = 38) = 3.98, P = 0.046$]. The race gap in self-reported health was eliminated in the treatment condition; interestingly, there was no gap for doctor visits (23). Future research should examine whether these patterns generalize to physiological and physical indicators of health (9) to assess the robustness of the effect beyond self-report outcomes and to identify biological pathways (11).

As further evidence of improved well-being, African Americans also scored higher on the Subjective Happiness Scale (23) [$t(35) = 2.61, P = 0.013$] (Fig. 4C). The happiness gap with European Americans higher than African Americans disappeared in the treatment condition (23). The finding of a lasting positive impact on subjective happiness is noteworthy in light of research showing that individual happiness is relatively stable (6).

Participants were unaware of the intervention's effect, suggesting that its efficacy did not depend on conscious awareness. Most students reported that they remembered participating in the study 3 years earlier (79% did). But when asked to describe "the most memorable and important" information they had learned in the study, few recalled the key content of the survey they had read (8% did), and few reported that the study had had "any" effect on their college experience (14% did) (table S3). There was no condition difference on any of these outcomes for African Americans [$\chi^2(1, N = 37 \text{ to } 38)$ values $< 1.40, P$ values > 0.20]; treated African Americans ascribed no more effect to the study than untreated African Americans. However, indirect measures of recall and beliefs did show treatment effects. When asked to "guess" the process of change described in the survey they had read, more treated than untreated African Americans wrote that it concerned how students' social experiences in college improve over time (50% versus 20%) [$\chi^2(1, N = 38) = 3.79, P = 0.052$]. Additionally, treated students endorsed this message. When asked to describe their own experiences, more treated than untreated African Americans volunteered that their own social experiences in college had improved over time (50% versus 20%) [$\chi^2(1, N = 38) = 3.79, P = 0.052$]. The subtle nature of this intervention, with its influence occurring outside conscious awareness (29), may contribute to its efficacy. In some cases, conscious awareness may undo the effects of an intervention (30). More overt interventions risk sending the stigmatizing message that the beneficiaries are seen as in need of help. They may also cause resistance and reactance and undermine the sense of accomplishment people take in their success (26).

This study provides an experimental, longitudinal demonstration that a brief intervention to buttress feelings of social belonging can have significant effects on a wide range of important outcomes. The social-belonging intervention im-

proved the academic performance, self-reported health, and well-being of ethnic minority students over 3 years. The results suggest that inequality between marginalized and nonmarginalized groups arises not only from structural factors but also from concern about social belonging.

This concern can be mitigated by using a psychological remedy. The intervention provided students a nonthreatening frame for interpreting the daily challenges of school. By encouraging students to adopt this message as their own, the intervention made this message stick psychologically. Along with other recent research, this study

highlights how the impact of adversity depends on its perceived meaning—how it is subjectively construed (24–26, 31–33). Changing subjective construal is a fruitful avenue for intervention because many events are ambiguous and amenable to multiple interpretations. Moreover, a change in construal can become self-reinforcing. Students who feel confident in their belonging may experience the social world in a way that reinforces this feeling. They may initiate more relationships and thus obtain more opportunities for belonging and growth. Brief interventions that shore up belonging can thus promote performance

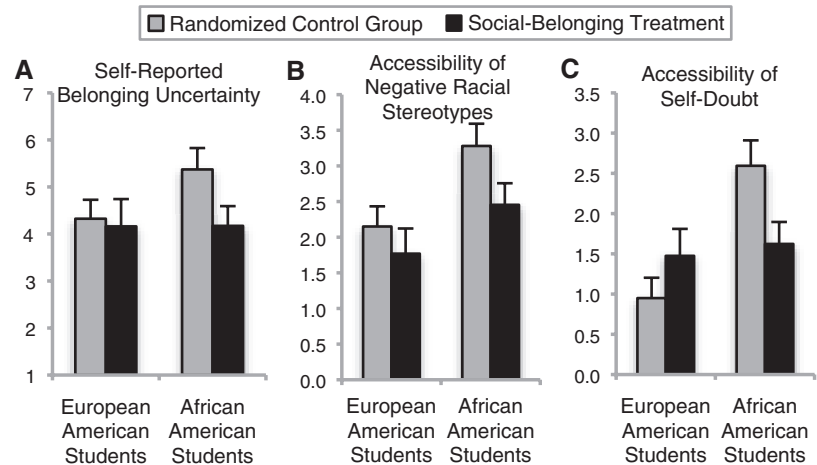


Fig. 3. Self-reported belonging uncertainty and the cognitive accessibility of negative racial stereotypes and of self-doubt 3 years postintervention. Error bars represent ± 1 SE. Data were combined across cohorts where measures were completed by both cohorts. (A) Self-reported belonging uncertainty (cohort 2). (B) Accessibility of negative racial stereotypes (cohorts 1 and 2). (C) Accessibility of self-doubt (cohorts 1 and 2). The y axis in (A) represents the full range of the scale. The y axes in (B) and (C) represent about 3.00 standard deviations. Sample sizes in cohort 2 only are $N_{\text{European Americans}} = 20$ and $N_{\text{African Americans}} = 23$. Sample sizes in cohorts 1 and 2 are $N_{\text{European Americans}} = 31$ and $N_{\text{African Americans}} = 38$.

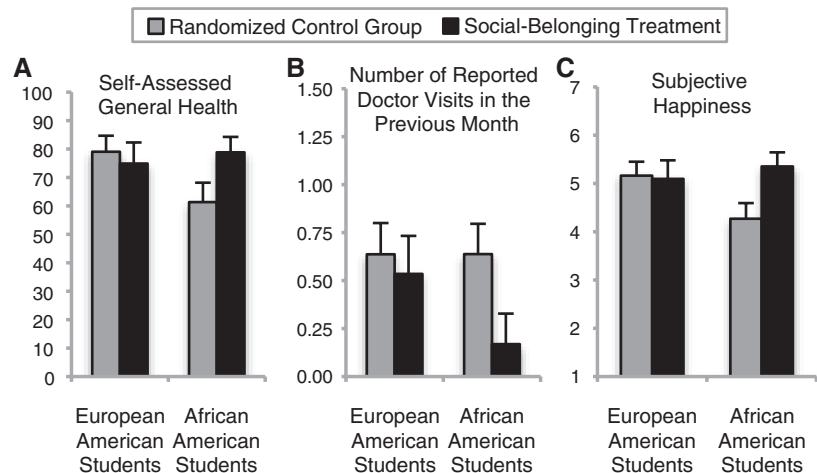


Fig. 4. Self-reported health and happiness 3 years postintervention. Error bars represent ± 1 SE. Data were combined across cohorts where measures were completed by both cohorts. (A) Self-assessed general health (cohort 2). (B) Reported doctor visits in the previous month (cohorts 1 and 2). (C) Subjective happiness (cohort 2). The y axes in (A) and (C) represent the full range of each scale. The y axis in (B) represents about 2.00 standard deviations. Sample sizes in cohort 2 only are $N_{\text{European Americans}} = 20$ and $N_{\text{African Americans}} = 23$. Sample sizes in cohorts 1 and 2 are $N_{\text{European Americans}} = 31$ and $N_{\text{African Americans}} = 38$.

and well-being even long after their delivery (7, 20, 25, 31, 34).

Importantly, the effectiveness of social-psychological interventions depends on factors in the context. Such interventions are unlikely to be effective in contexts without opportunities for learning. Also, because the present intervention works by changing people's subjective interpretation of ambiguous events, it may be ineffective in openly hostile environments. Lastly, whether this intervention would work among younger or less-select students, or students from other marginalized groups, is an important question for future research (20, 31, 34). These qualifications noted, the results underscore the importance of social belonging and subjective construal in contributing to social inequality and show how this insight can inform our collective efforts to promote equality in performance, health, and well-being.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/331/6023/1447/DC1
Materials and Methods
Tables S1 to S3
References

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Direct Interaction of RNA Polymerase II and Mediator Required for Transcription in Vivo

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Gene transcription is highly regulated. Altered transcription can lead to cancer or developmental diseases. Mediator, a multisubunit complex conserved among eukaryotes, is generally required for RNA polymerase II (Pol II) transcription. An interaction between the two complexes is known, but its molecular nature and physiological role are unclear. We identify a direct physical interaction between the Rpb3 Pol II subunit of *Saccharomyces cerevisiae* and the essential Mediator subunit, Med17. Furthermore, we demonstrate a functional element in the Mediator–Pol II interface that is important for genome-wide Pol II recruitment in vivo. Our findings suggest that a direct interaction between Mediator and Pol II is generally required for transcription of class II genes in eukaryotes.

Mediator is a large multisubunit complex conserved in all eukaryotes (1). It acts as a link between specific protein regu-

lators and the RNA polymerase II (Pol II) transcription machinery (2). Mediator is required at most Pol II–transcribed gene promoters for regulated gene expression (3–5). In *Saccharomyces cerevisiae*, Mediator is composed of 25 subunits and is organized in four structural modules: the tail, middle, head, and Cdk8 modules (6). A direct Mediator–Pol II interaction is indicated by previous copurification, coimmunoprecipitation (CoIP) experiments (7–9) and by in vivo formaldehyde cross-linking (10). A model of the Mediator–Pol II complex determined by electron

microscopy (EM) at 35 Å resolution suggests that several Pol II subunits (Rpb1, 2, 3, 6, and 11) might contact the middle or the head of Mediator (11). It was recently suggested that Rpb4 and Rpb7 could also be implicated in interactions with Mediator (12–14). However, the requirement of a direct interaction between Mediator and Pol II for transcription activation has not been demonstrated. Moreover, the identity of the Mediator subunits contacting Pol II is unknown because of the low resolution of the Mediator structure. As a consequence, the mechanism by which Mediator recruits Pol II is poorly understood.

To identify the subunit(s) of Mediator that directly contact Pol II and determine the role of these interactions in transcription regulation, we used an in vivo photo-cross-linking approach based on the incorporation by the cell-translation system of photo-activable analogs of methionine and leucine in proteins [see supporting online material (SOM) text and figs. S1 and S2] (15, 16).

Because EM results (11) suggested potential interactions of 16 Mediator subunits belonging to the head (Med6, 8, 11, 17, 18, 19, 20, 22) and middle (Med1, 4, 5, 7, 9, 10, 21, 31) modules with Rpb1, 2, 3, 6, or 11 Pol II subunits, we immunoprecipitated hemagglutinin (HA)-tagged proteins after in vivo cross-linking. Among the 80 pairwise contacts that we tested, only Myc-tagged Rpb3 and HA-tagged Med17 cross-linked (Fig. 1). These results demonstrate that the Rpb3 Pol II

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subunit directly interacts with the essential Med17 subunit of the Mediator head module, in line with the peripheral location of Rpb3 in the Pol II structure (17, 18) and with the general requirement for Med17 in transcription (4).

A thermosensitive *rpb3-2* mutant defective in activator-dependent transcription in vitro and in vivo was described previously by Tan *et al.* (19). The mutation contains two amino acid substitutions [Cys⁹²→Arg⁹² (C92R) and Ala¹⁵⁹→Gly¹⁵⁹ (A159G)] (20) that have a strong synergistic effect on the thermosensitive phenotype. We mapped the mutated amino acid residues of *rpb3-2* on the crystallographic structure of Pol II. C92 is located on the subunit surface, and A159 is close (~7 Å) to C92 (Fig. 2A). We tested whether the Med17-Rpb3 contact is modified in the *rpb3-2* mutant. The in vivo photo-cross-linking approach could not be used in the *rpb3-2* mutant, because the incorporation of the photo-amino acids was strongly diminished due to the mutant slow growth. We turned to a formaldehyde cross-linking strategy and detected Rpb3-Med17 cross-linking in the wild-type (WT) strain (Fig. 2B). This contact was strongly reduced in the *rpb3-2* mutant. CoIP experiments with chromatin and soluble extracts further confirmed that Mediator-Pol II association was impaired in the *rpb3-2* mutant (see SOM text and figs. S3 and S4).

Previous results have shown that the mRNA level of the *GAL1*-inducible gene was decreased in the *rpb3-2* mutant (19). We therefore examined Pol II and Mediator occupancy on the *GAL1* gene by chromatin immunoprecipitation (ChIP) experiments after galactose induction. Pol II occupancy of the *GAL1* gene was significantly reduced in the *rpb3-2* mutant compared with the wild type (Fig. 2C). In contrast, Mediator occupancy of the *GAL1* promoter in the *rpb3-2* mutant was similar to the WT level with only a marginal reduction after a 45-min induction. As for the *GAL1* gene, a clear decrease of Pol II occupancy can be observed in the *rpb3-2* mutant, but not for Mediator association to the *ADH1* and *PYK1* genes that are constitutively expressed (fig. S5). Our results suggest that the *rpb3-2* mutant is affected in the Mediator-Pol II interaction leading to defective Pol II recruitment, even though other factors could also contribute to this defect. To investigate whether the decrease of Pol II occupancy in the *rpb3-2* mutant affects transcription in general, we analyzed the genome-wide Pol II occupancy by ChIP followed by DNA hybridization on microarrays. Regression analysis of Pol II binding in the WT versus *rpb3-2* mutant revealed a global 1.5-fold decrease in the mutant with a high correlation coefficient (Fig. 2D), suggesting that Med17-Rpb3 interaction is required for the recruitment of Pol II on most active genes.

To investigate the in vivo role of Med17 in Pol II recruitment, we selected 29 *med17* thermosensitive mutants and showed that 5 of them (*med17-68*, -158, -208, -257, and -327) were lethal with the *rpb3-2* mutation (Fig. 3A and fig. S6A). In a *RPB3* WT background, these five *med17* mu-

tants had diverse slow-growth phenotypes at 30°C (fig. S6B). The allele-specific lethality between the *rpb3-2* and *med17* mutations is consistent with a physiological role for the Med17-Rpb3 interaction.

To analyze the molecular nature of the *med17* thermosensitive mutations showing specific synthetic phenotypes in *rpb3-2* background, we determined the Pol II and the Mediator occupancy in the *med17-68*, -208, and -257 mutants by ChIP experiments in a *RPB3* WT background. A strong decrease in Pol II association with the *ADH1* and *PYK1* genes was observed in these mutants compared with the wild type (Fig. 3B). In contrast, the Mediator association (measured by the Med5 middle, Med8 head, or Med15 tail subunit ChIP) to the *ADH1* and *PYK1* gene promoters was similar to the WT level (fig. S6, C, D, and E). The integrity of Mediator in *med17* mutants has been verified by CoIP experiments (fig. S7).

Using random mutagenesis of *MED17*, we looked for *med17* mutants that would suppress the growth defects of *rpb3-2* (C92R A159G) at 37°C. No suppressor was obtained, but we isolated several *med17* mutants that suppressed the mild thermosensitive defect of *rpb3-A159G* single mutant (Fig. 4A). One representative suppressor mutant, *med17-sup1* (I128V R582G N595D), was chosen for further analysis. We determined the effect of *med17-sup1* mutation on Pol II and

Mediator occupancy in the *rpb3-A159G* background. Figure 4B and fig. S8 show that the association of Pol II to the *ADH1*, *PYK1*, and *PHO84* genes is slightly but significantly decreased in the *rpb3-A159G* mutant and is restored to WT levels in the *rpb3-A159G med17-sup1* strain. The Mediator association to the *ADH1*, *PYK1*, and *PHO84* promoters remained at WT levels (fig. S8B). As for Pol II, the association of the general transcription factor TFIID was also diminished in the *rpb3-A159G* mutant, and the *med17* suppressor restored near WT TFIID occupancy (fig. S8C).

MED17 was initially isolated as *SRB4*, in reference to mutations that suppressed the growth phenotype of truncations of the Pol II Rpb1 C-terminal repeat domain (CTD) (9). The *med17* suppressors were unable to compensate the conditional-growth phenotypes of the *rpb1-Δ104* CTD-truncation mutant (fig. S9), indicating that the suppression is specific for *rpb3-A159G*.

A functional link between Mediator and Pol II CTD was proposed after the identification of suppressors of CTD-truncation mutants affecting the Mediator subunits (9, 21), because Mediator plays a stimulatory role in CTD phosphorylation by TFIID (7, 22) and binds to glutathione *S*-transferase-CTD (23). However, a low-resolution model of the Mediator-Pol II complex suggests that, beyond its presumed interaction with CTD,

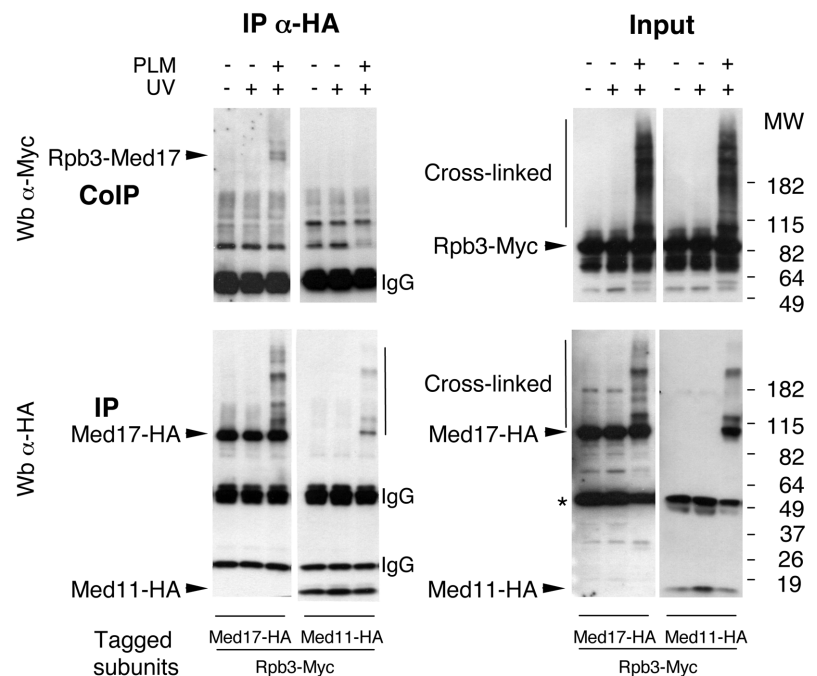


Fig. 1. Direct in vivo interaction of the Rpb3 Pol II subunit with the Med17 Mediator subunit. Rpb3-Myc Med17-HA or Rpb3-Myc Med11-HA strains grown in the presence or absence of photo-leucine plus photo-methionine (PLM) were ultraviolet (UV)-irradiated or not, as indicated. Proteins were immunoprecipitated (IP) with α -HA antibody from crude extracts (Input). Immunoprecipitated or coimmunoprecipitated (CoIP) proteins were analyzed by Western blotting (Wb) with α -HA or α -Myc antibodies, respectively. The cross-linked Rpb3-Med17 band is indicated. The position of unidentified cross-linked proteins with the tagged Mediator or Rpb3 subunits is indicated by a vertical bar. The position of the proteins and immunoglobulin G (IgG) heavy or light chains is indicated. An asterisk marks a nonspecific band. MW, molecular weight in kilodaltons.

Fig. 2. The *rpb3-2* mutant is defective in Mediator-Pol II association and Pol II occupancy. **(A)** Positions of the residues (A159, C92) mutated in *rpb3-2*. A ribbon model of the yeast Pol II structure (Protein Data Bank code 1WCM) is presented. Pol II subunits are shown in gray, except for Rpb3, which is shown in orange. The blue spheres mark A159 and C92. A box zooms in on the surface of Rpb3 with C92 in blue. A159 is located beneath the subunit surface and is thus invisible on the enlargement. To focus on the Rpb3 surface, the structure in the box was rotated. **(B)** Med17-Rpb3 contact is affected in the *rpb3-2* mutant. Rpb3-Myc Med17-HA or *rpb3-2*-Myc Med17-HA strains were cross-linked (or not) with formaldehyde (FA), as indicated. Med17-HA was immunoprecipitated with α -HA antibody from crude extracts (Input) and analyzed by Western blotting with α -Myc antibody (CoIP) against Rpb3. The cross-linked Rpb3-Med17 band is indicated in red. The position of unidentified cross-linked proteins with the tagged Med17 or Rpb3 subunits is indicated by a vertical bar. An asterisk marks a nonspecific band. **(C)** Pol II and Mediator occupancy on the *GAL1* gene upon galactose induction. The *rpb3-2* and WT strains were grown in a raffinose-supplemented medium, then galactose was added for 45 or 120 min. ChIP was performed with a α -Rpb1 antibody (Pol II) and with a α -HA antibody against Med17-HA (Mediator). Mean values and SDs (indicated by error bars) of three independent experiments are shown. The location of the polymerase chain reaction (PCR) fragments is indicated (P1, O1, O2). A control corresponds to a nontranscribed region on chromosome V. IP/IN, ratio of

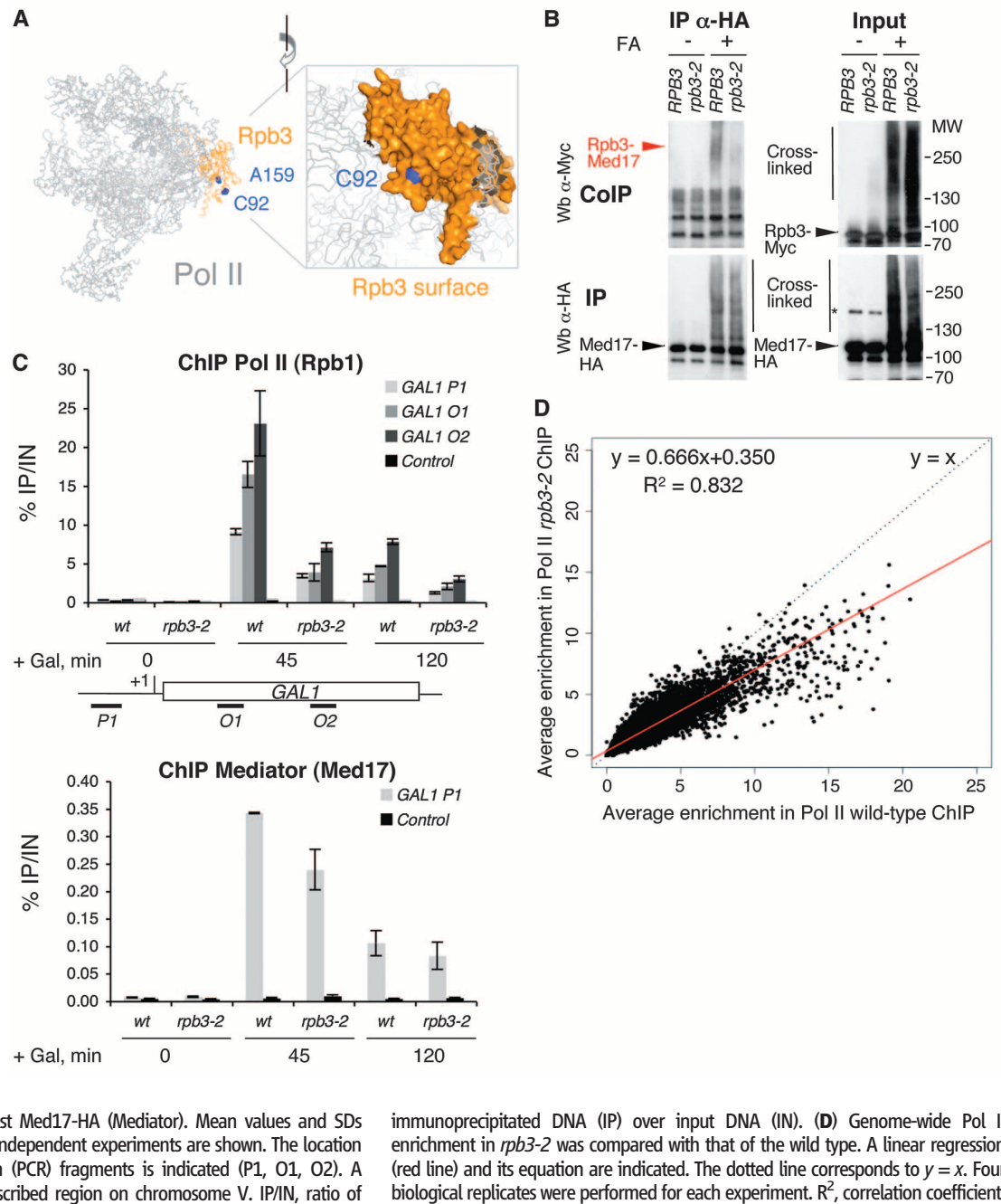


Fig. 3. Specific *med17* thermosensitive mutations are lethal with *rpb3-2* and show severe Pol II occupancy defects. **(A)** *med17* thermosensitive mutations are lethal with *rpb3-2*. Cells were serially diluted, spotted on yeast extract, peptone, and dextrose (YPD)—or 5-FOA-containing agar plates (to counterselect the WT *MED17*-bearing plasmid), and incubated at 30°C for 7 days. **(B)** Cells were grown at 30°C. ChIP was performed with a α -Rpb1 antibody for Pol II. Mean values and SDs (error bars) of three independent experiments are shown. The *GAL1* open reading frame (ORF) was used as a control.

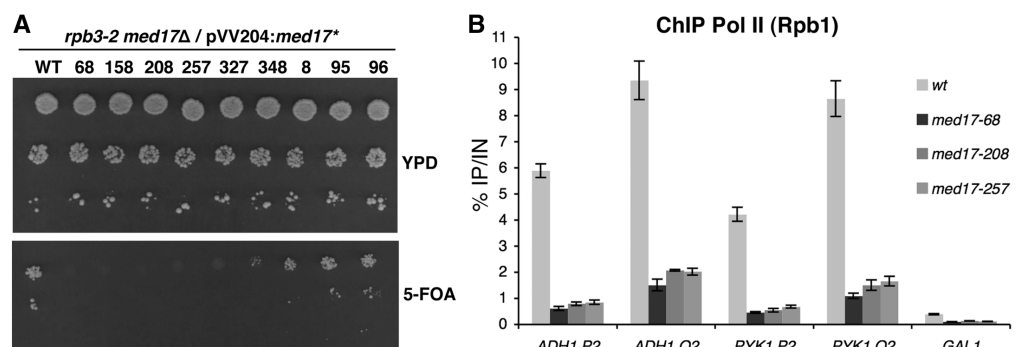
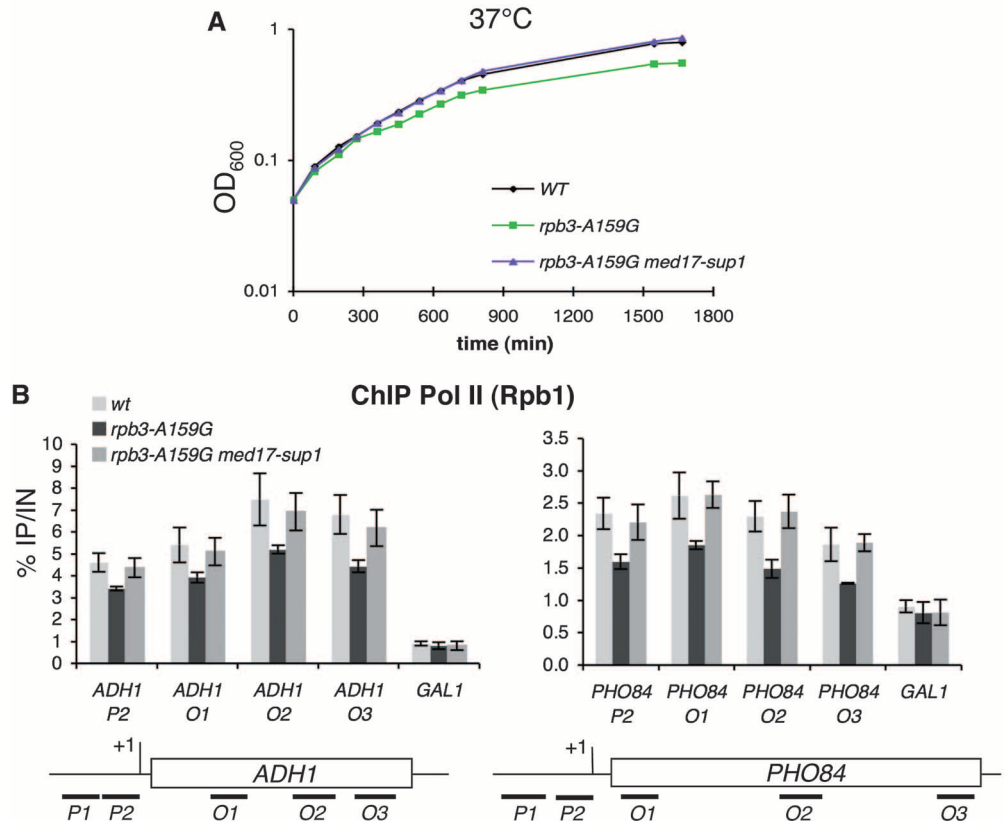


Fig. 4. The *med17* mutation suppresses the *rpb3-A159G* growth phenotype and Pol II–association defects. **(A)** The *med17-sup1* mutation suppresses the *rpb3-A159G* growth phenotype. Cells were grown in a liquid YPD medium at 30°C and then shifted to 37°C at time 0 min. The optical density at 600 nm (OD_{600}) is indicated on the y axis. The growth curves for WT strain, *rpb3-A159G*, and *rpb3-A159G med17-sup1* mutants are shown in black, green, and blue, respectively. **(B)** Cells were grown at 30°C and then shifted to 37°C for 5 hours. ChIP was performed with a α -Rpb1 antibody for Pol II. Mean values and SDs (error bars) of three independent experiments are shown. The *GAL1* ORF was used as a control. The location of the PCR fragments is indicated (P1, P2, O1, O2, O3).



Mediator might engage in multiple contacts with the enzyme (17). Here, we have shown that Med17 interacts with Rpb3, and we selected *med17* suppressors specific for *rpb3-A159G* that had no *SRB* phenotype. Further, we observed allele-specific lethality between *rpb3-2* and *med17* thermosensitive mutations. The cross-linking approach that we used probably revealed only a subset of in vivo interactions. Indeed, we did not test all potential contacts between subunits of Mediator and Pol II, and we used only two photo-activable amino acids. Thus, it is possible that other contacts exist between the Mediator complex and Pol II.

We showed that the Rpb3 subunit plays an essential role in Pol II recruitment to most class II genes and that the Med17 Mediator subunit function is central to preinitiation complex formation. A direct contact between Med17 and Rpb3 that is structurally homologous to the bacterial α subunit implicated in transcription activation (19, 24) further supports the apparent conservation of the RNA polymerase surface involved in transcription regulation from prokaryotes to eukaryotes, even though the molecular mechanisms and interacting partners differ.

Our results support the notion that the direct interaction of the Rpb3 Pol II subunit with the Med17 Mediator subunit is essential for global Pol II recruitment in vivo. Therefore, Mediator might be considered a general transcription factor (25). In addition, with the conservation of Pol II (26) and Mediator (1), the mechanism of transcription activation by Pol II recruitment through

the contact between Rpb3 and Med17 may extend to all eukaryotes.

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Supporting Online Material

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MOLECULAR AND SYSTEMS BIOLOGY in Cardiovascular and Metabolic Diseases at UCLA

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RECOVERING FROM POSTDOC MISTAKES

"The best thing you can do in a postdoc is to do things that you enjoy," urges **Doon Gibbs**, deputy director for science and technology at Brookhaven National Laboratory, who has overseen the supervision of many postdocs over 25 years. Ensure those tasks are noticeable, such as publishing papers and presenting at conferences, he adds. But possessing a passion and self-promotion prowess alone does not a successful scientist make. Too often postdocs end up making mistakes along the way that can sideline them from the vocation they desire. Whether it is spending too long in a postdoc appointment, relying too much on their advisor, or simply not taking ownership of their career, there are many possible ways that early-career scientists can blunder. But luckily, there are clever means and methods to remedy even the most serious of slip-ups.

By Alaina G. Levine

"It is vitally important for postdocs to be aggressive and take charge of their careers."



Jimmy Weterings



Doon Gibbs

WHY AM I HERE?

The postdoc appointment is meant to serve as the stepping-stone to victory in academic science and certain positions in industry, says **Harold Myron**, former director of education programs at Argonne National Laboratory. The job is designed as a training program to instill certain skills, techniques, tools, and tactics for pursuing advanced research. Ideally, a postdoc should sharpen their innovative problem-solving abilities and learn to manage research group resources, such as employees and grant money.

Too often graduate students take a postdoc appointment for the wrong reasons, which of course, can be a mistake in and of itself. There is a decades-old tradition that "the postdoc is a training ground for a tenure-track position, that this is the metric for success for young scientists," says **Cathee Phillips**, executive director of the National Postdoctoral Association (NPA). "Postdocs have heard this for years, which causes them not to think about their own strategic career plan, because they think the postdoc will naturally flow into a tenure-track position." But with only 20 percent of postdocs advancing to tenure-track employment, many find themselves realizing too late, or waiting too long, to make a career plan with tangible contingency options.

Sebastian (not his real name), who works as an administrator at a medical school in the southern United States, admits he made a number of mistakes in his two postdocs, not the least of which were going in naively and staying too long without a concrete career plan. "If you don't want a tenure-track position, then there's no reason to do a postdoc," he declares, and reveals that his lack of planning led to miscommunication with his bosses and wasted time.

In his first appointment, which only lasted a year, Sebastian's principal investigator (PI) needed someone experienced in a particular biomedical technique, which he did not have. The mentor did not have time to train his protégé, which led to arguments. "It was the wrong lab for me, and my naivety led me to accept bullying [from my advisor]," he says. "My mistake was that I stayed as long as I did."

Ultimately, Sebastian recovered from what could have been a

costly career blunder by forging his own path into academic program management. "It is vitally important for postdocs to be aggressive and take charge of their careers," he cautions.

ALL THE DOCTORATES ARE DOING IT...

When deciding whether to accept a particular postdoc, it is paramount to confirm that you are proceeding with the appointment for the right reasons.

However, frequently, graduate students venture into a postdoc out of a feeling of desperation for a job, resulting in a lack of inquiry about basic elements of the appointment and little or no negotiation for benefits. "When you're finished with your Ph.D., people look into what postdocs they want," confesses **Jimmy Weterings**, whose appointment took place at Vrije Universiteit Amsterdam in the Netherlands, and who is currently seeking an academic position. "There are some people, and I count myself among them, who will take anything—it's a safety feeling. You finished your Ph.D., you know you will have income, but I didn't think beyond the two years."

Weterings, who did not possess a strategic plan, neglected to bargain for essentials that would have bolstered his **continued »**

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POSTDOC POSITIONS



“Go home every night and ask yourself ‘what did I accomplish today that furthered my career?’ If every day you accomplish nothing, you need to take action...it’s a warning sign of bad things.”

—Trevor Penning

career progression, such as teaching his own course. “In hindsight, I learned a lot about negotiation and talking with people,” he says. Weterings advises you can prevent this common mistake by relinquishing the feeling of desperation that you have to take a job without asking vital questions about it from the start. And “don’t take the first one that comes along,” he adds. “Think if it’s the right opportunity, ask yourself ‘what will I accomplish?’”

Fiona (not her real name), who recently finished a two-year biology postdoc at a state university in the western U.S., had been thinking of leaving academia after earning her Doctorate. She decided to pursue the postdoc anyway because finishing her thesis left her “on a high,” and she thought “it might be rash” to depart the tenure-track too soon. Her gaffe was that she did “not question people enough” about what she would be doing day-to-day. “I was so excited about getting a job,...I didn’t think to ask how people in the lab generally felt,” she says.

Within the first year of her appointment, her project lost funding. There was no financial support to attend conferences. And with a PI who was close to retirement and on sabbatical, Fiona discovered difficulty maintaining motivation.

But Fiona was lucky in one respect: Although she did not have a crystallized career plan when she entered her postdoc, she did have ideas about where she wanted to go next. She leveraged her postdoc experience to launch a fulfilling career in medical writing.

HELP! I’M IN A POSTDOC AND CAN’T GET OUT!

Although the number of years one spends in a postdoc differs depending on the chosen field, specialty, and career choice, most advisors agree that three to five years should be the cut-off point. Yet, some postdocs stay much longer, languishing with seemingly no end in sight—a big mistake, stress some experts. “The postdoc experience is not meant to be limitless,” says **Trevor Penning**, who served as associate dean for postdoctoral research and training at the University of Pennsylvania School of Medicine from 1997 to 2005.

Some stay because they don’t know what else to do. “Knowing you have x years, a postdoc must develop a timeline and goals,” continues Penning. “Go home every night and ask yourself ‘what did I accomplish today that furthered my career?’ If every day you accomplish nothing, you need to take action...it’s a warning sign of bad things.”

With improper planning and a lack of assertiveness, early-career scientists can get stuck in a seemingly endless hamster wheel of postdoc appointment after appointment. After not landing a tenure-track position the first or second time around, some postdocs simply stay put where they are while others pursue another postdoc.

Gibbs is concerned that a postdoc who stays too long could be

LESSONS LEARNED

- Have a Strategic Plan
- Have a Plan B
- Be aware that tenure-track positions are hard to come by
- Don’t go into a postdoc just because you don’t know what else to do
- Have more than one mentor
- Be wary of a mentor who is absent, on sabbatical, or runs a large lab
- Don’t burn your bridges
- Communicate with your mentor
- Postdoc positions are a two-way street with you and your mentor
- Make use of your university’s postdoc office
- Apply for your own funding
- Look for a place that offers professional development
- Ensure you accomplish noticeable tasks during your postdoc, such as publishing papers and presenting at conferences

RESOURCES

National Postdoctoral Association
www.npa.org

PostDoc Forum
www.postdocsforum.com

Science Careers Forum
http://scforum.aaas.org

taken advantage of by their supervisor. Sebastian for example, feels he was treated as a technician during his postdoc. According to other associates and even PIs, it is not uncommon for some postdocs to be looked upon as an extra pair of hands and be charged with less complex routine tasks, as opposed to more creative, scientifically driven endeavors.

“If you find yourself in a situation that is untenable, [ask yourself] ‘is it in my best interest to stay in the lab?’” suggests Penning. Identifying the problem early is critical. “It’s much better to lose one year than five.”

THE MEDIUM IS ME (AND MY MENTOR)

Your mentor has the potential to heavily influence your career. But it is *your* career. Mary (not her real name), who received her Ph.D. in the biological sciences, proffers serious counsel regarding the all-too-frequent misstep of allocating complete control of your livelihood to another person, especially your supervisor. “Never expect your mentor to only be looking out for you. You have to look out for yourself,” she says. After all, “your boss’s priority is their own career.”

There is so much riding on your relationship with your PI, so “choose your postdoc mentor carefully,” warns Mary. In addition to serving as your advisor, and ideally as a coach and **continued »**

Janelia Junior Fellows Program



The Howard Hughes Medical Institute's Janelia Farm Research Campus is a world-class biomedical research center where outstanding scientists from diverse disciplines use emerging and innovative technologies to pursue biology's most challenging problems.

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Photo by Paul Fettes

POSTDOC POSITIONS



“When you ask for help, if help is not forthcoming, your decision is easy: you find another mentor.”

—Philip Clifford

FEATURED PARTICIPANTS

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Brookhaven National
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Medical College of
Wisconsin
www.mcw.edu

National Postdoctoral
Association
www.nationalpostdoc.org

University of Pennsylvania
School of Medicine
www.med.upenn.edu

Vrije Universiteit
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www.vu.nl/en

champion, a good mentor should help orchestrate pathways for you to advance to the next stage of your career. So examine his or her track record of training associates, and pick “someone who moves people on to successful positions,” she says.

Mary made the mistake of not checking on this before securing her appointment. Her PI had never gotten anyone through a Ph.D., let alone on to a good job, she claims. As such, her career has progressed at a very slow pace. She is now in her second postdoc at the same institution where she received her Doctorate.

“Postdocs have to be realistic about what to expect from their mentors,” says Phillips. “These are busy people, and just because they hired postdocs doesn’t mean they will automatically be good mentors, particularly if you are looking at a career outside of academia.” Moreover, “the [appointment] is a two-way street with the mentor,” notes Penning, “but the postdoc has to take ultimate responsibility for their own career success or failure.”

PEDIGREE IS NOT ALWAYS KEY

Another classic conundrum is the sometimes misguided conviction that in order to progress in academe, you must spend your postdoc in a big, well-funded research group, says Carla (not her real name), a biologist who completed a five-year postdoc at a prestigious private university. But a large lab run by a famous scientist doesn’t guarantee direct value for the postdoc, as she found out the hard way.

Carla, who is an assistant professor at a medical school in the eastern U.S., divulges that her postdoc was complicated by lack of face time with her PI. The supervisor was well-known and traveled extensively. He directed an enormous lab, consisting of a score of postdocs who were all jockeying for time with their advisor. Upon returning from his trips, the PI “would only talk to those whose projects meant the most to him or to people close to submitting a paper,” she says.

But her research was not the group’s main focus, so she did not receive substantial opportunity to meet with her mentor. Carla recommends staying on the supervisor’s “radar screen” by making a careful decision to work on a project that is the highlight of the lab, she says.

“The largest labs may not give you the skills to be a professional scientist,” warns Penning. And selecting your mentor “is not just about the lab’s pedigree,” he adds. “Choosing the wrong person to be a mentor can make an experience go south from the beginning.”

But by networking and seeking out other mentors besides their PI, a postdoc can remedy a difficult situation. “They can’t depend only on the PI for points of contact,” says **Philip S. Clifford**, associate dean of the Graduate School of Biomedical Sciences at the Medical College of Wisconsin.

If there is confusion as to whether your supervisor is actively participating in your professional advancement, “it boils down to good communication between the mentor and the postdoc,” states Penning. “When you ask for help, if help is not forthcoming, your decision is easy: you find another mentor.”

WHEN ANOTHER POSTDOC TAKES OVER

There is great internal competition among postdocs that is often not acknowledged, admits Carla. She describes how another person in her lab “liked my project and usurped it,” and the PI, whose management skills were subpar, did nothing. Carla was then faced with the decision of whether to leave the lab, start something fresh, or partner with someone who seemed like the enemy. “I decided to collaborate,” she recalls, “but I ended up suffering because the other person ended up talking about it on job talks,” which she felt limited her ability to use it in presentations for academic positions.

Carla’s solution was to recognize that there was enough room in the job market and in the research field for her to differentiate herself from the other party. But “the onus was on me to distinguish myself,” she says. “I took the hard road, but in the end, this gives the most meaning in science.”

If you find yourself in a situation where you are faced with an internal rivalry that could backfire, Carla suggests speaking with the PI and the other person to find ways to partner together. For example, there might be an angle that allows both people to co-first-author a manuscript. Communication is crucial, she says, “so everyone maxes out the benefits.” The bottom line is you don’t want to burn any bridges.

THE PORTAL TO SUCCESS

Every vocation has potential pitfalls and every professional has made their share of mistakes. Whether it’s spending too much time in “PostdocLand,” choosing the wrong mentor or lab, or not having a targeted career plan with flexibility for unforeseen twists, there will always be opportunity to err in academic science. Fortunately, as sources say, if you recognize that you are in the driver’s seat, acknowledge a problem’s existence early on, and focus on finding a resolution, you can recover and discover success. There are plenty of resources to aid you on your adventure, (see “It Pays to Plan: Why You Need a Career Map,” DOI:10.1126/science.opms.r1000098), and best of all, if you learn from your mistakes, some might argue they were never mistakes in the first place. As James Joyce wrote, “A man of genius makes no mistakes. His errors are volitional and are portals of discovery.”

Alaina G. Levine is science writer based in Tucson, AZ.

DOI: 10.1126/science.opms.r1100101



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LUXEMBOURG'S RESEARCH PROGRAMME FOR
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If you are an **internationally recognised senior researcher**, our **research programme PEARL** gives you the opportunity to transfer your research programme to a public-sector research institution in Luxembourg and thus to accelerate the development of and to strengthen Luxembourg's research priorities. 3-5M€ are offered to Luxembourg's public-sector research institutions through this programme to compete for the best candidates. The FNR foresees to grant 1 to 2 PEARL awards per year. The call is open all year.



LUXEMBOURG'S RESEARCH PROGRAMME FOR OUTSTANDING
YOUNG RESEARCHERS FROM ALL OVER THE WORLD

If you are an **outstanding young researcher**, our **research programme ATTRACT** helps you to set up an independent research team within a public-sector research institution in Luxembourg. The innovation, dynamism and creativity of your project as well as its high scientific quality should enhance Luxembourg's position in the international world of R&D. Projects selected under ATTRACT have a lifespan of five years and the financial contribution will be up to 1.5M€. The 6th ATTRACT Call will be launched in December 2011.

More information about ATTRACT and PEARL as well as the other funding opportunities offered by the National Research Fund Luxembourg can be found on the FNR's website. Go and see what's behind on www.fnr.lu



Fonds National de la
Recherche Luxembourg

INVESTIGATING FUTURE CHALLENGES

*iCrea

INSTITUCIÓ CATALANA DE
RECERCA I ESTUDIS AVANÇATS

CATALAN INSTITUTE FOR
RESEARCH AND ADVANCED STUDIES

Senior Research Positions 2011

ICREA announces the opening of **20 senior research positions** in all fields of research.

Minimum requirements are a Ph.D. degree, obtained preferably before 2007, and four years of international exposure at the doctoral and/or post-doctoral level. **Only those candidates with an outstanding research record and excellent leadership capabilities will be considered.**

Successful applicants will have a **permanent contract** with ICREA and will work at universities or research centres in Catalonia. Salaries will be in line with those paid at Catalan universities.

ICREA research professors will be subject to an evaluation of research progress and general performance after a three-year period, and subsequently every five years. A positive evaluation will lead to a salary increase.

For further details visit www.icrea.eu.

Applications and deadline

Applications must be submitted electronically via ICREA's website www.icrea.eu. The website provides all the information needed to apply. **Deadline:** May 2, 2011

***ICREA** is a foundation sponsored by the Ministry of Economy and Knowledge of the Government of Catalonia (*Generalitat de Catalunya*). ICREA's main goal is to promote high-level research in Catalonia.



MAX-PLANCK-INSTITUT
FÜR DEMOGRAFISCHE
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FOR DEMOGRAPHIC
RESEARCH

The Max Planck Institute for Demographic Research seeks

12 doctoral students, post-docs and junior research scientists in Demography, Biology, Statistics and Mathematics

to join 28 researchers from diverse fields doing research on humans and on animals and plants across the tree of life to develop

Evolutionary Biodemography.

We are looking for creative minds eager to work on interdisciplinary teams to tackle bold new research questions.

Please see www.demogr.mpg.de/jobs.



MAX-PLANCK-GESellschaft

Wellcome Trust-MIT Postdoctoral Fellowships

This scheme offers opportunities for postdoctoral scientists to undertake research at the interfaces between biology/medicine and mathematics, engineering, computer, physical or chemical sciences, firstly at MIT and then at a UK institution.

Candidates will be expected to identify an important biomedical research question and to propose a personal interdisciplinary training programme to achieve their research aims.

Deadline for submission of a full application is 1 July 2011.

www.wellcome.ac.uk/mit/science



wellcometrust

UAB THE UNIVERSITY OF ALABAMA AT BIRMINGHAM

Postdoctoral Positions

The University of Alabama at Birmingham (UAB) is one of the premier research universities in the US with internationally recognized programs in AIDS and bacterial pathogenesis, bone biology and disease, cancer, diabetes and digestive and kidney diseases, free radical biology, immunology, lung disease, neuroscience, trauma and inflammation, and basic and clinical vision science among others. UAB is committed to the development of outstanding postdoctoral scientists and has been consistently ranked in recent years as one of the top locations among US universities for training postdoctoral scholars.

UAB is recruiting candidates for postdoctoral positions in a variety of research areas. UAB faculty are well funded (top 20 in NIH funding), utilize multidisciplinary approaches, and provide excellent research training environments that can lead exceptional candidates to entry level positions in academia, government or the private sector. Full medical coverage (single or family), competitive salaries/stipends, sick leave, vacation, and maternity/paternity leave are offered with every position as well as AD&D, disability and life insurance. Depending on the source of funding, retirement benefits may also be available. Birmingham is a mid-size city centrally located in the southeast near beaches and mountains and enjoys a moderate climate for year round outdoor activities and a cost of living rate lower than most metropolitan areas.

Visit our web site at www.postdocs.uab.edu, under Postdoctoral Opportunities to view posted positions. Send your CV and cover letter to the contact name for those positions for which you are qualified and which interest you. University of Alabama at Birmingham, Office of Postdoctoral Education, 205-975-7020.

UAB is an Equal Employment Opportunity Employer.

POSITIONS OPEN

Lecturer Marine Sciences

Stony Brook University's School of Marine and Atmospheric Sciences (SoMAS) seeks to hire a full-time lecturer with expertise in marine vertebrates including marine mammals to support our Bachelor of Science degrees in Marine Vertebrate Biology, Marine Sciences, and Environmental Studies. For a full position description, application procedures, or to apply online, visit www.stonybrook.edu/jobs (Job Reference #: F-6711-11-02) or submit a State employment application with cover letter, curriculum vitae, a statement of teaching philosophy, and contact information for three references in one document (electronic submission of materials is strongly preferred), via www.stonybrook.edu/jobs, Category A, Job Reference #: F-6711-11-02. Alternatively, please submit materials to: Dr. Christopher Gobler, Search Chair, School of Marine and Atmospheric Sciences, 145 Discovery Hall, Stony Brook University, Stony Brook, NY 11794-5000.

Stony Brook University/SUNY is an equal opportunity, affirmative action employer.



POSTDOCTORAL OPPORTUNITIES



GERMAN CANCER RESEARCH CENTER IN THE HELMHOLTZ ASSOCIATION

The German Cancer Research Center is the largest biomedical research institution in Germany. With almost 2,300 employees, we operate an extensive scientific program in the field of cancer research.

The Division of **Molekulare Neurobiologie** (Kst. G381) is seeking

Two Postdoctoral Positions (m/f) (RNA Biology or Mathematics)

(Ref-No. 22/2011)

Description: The division Neurobiology of Brain Tumors seeks two scientifically dedicated applicants to study the role of local RNA translation in axonal regeneration and stem cell differentiation. A genome wide transcriptome, polysome-bound RNA profile and methylation will be used for identification of factors involved in axonal growth and "fate specification" of stem cells. Prediction of RNA recognition motifs (RRMs), processing of next generation sequencing data, implementation of mathematical models and experimental validation of the data is planned.

Your profile: Applicants should hold a PhD in biology or related fields or alternatively in physics, mathematics or related fields. Applications should include cover letter, CV, copies of university exams and address details of 2 referees.

The position is limited for 2 years.

For further information please contact Dr. Ana Martin-Villalba, phone 06221 42-3766.

The German Cancer Research Center is committed to increase the percentage of female scientists and encourages female applicants to apply. Among candidates of equal aptitude and qualifications, a person with disabilities will be given preference.

Please send your application to

Deutsches Krebsforschungszentrum
Personalabteilung
Im Neuenheimer Feld 280
D-69120 Heidelberg

Or apply preferably online:
www.dkfz.de



POSITIONS OPEN

Senior Faculty Positions



The Center for Immunology and Microbial Disease at Albany Medical College invites applications for senior tenure-track faculty positions from individuals with internationally-recognized research programs in microbiology and/or host-pathogen interactions. The basic science departments at Albany Medical College are organized as interdisciplinary research centers and the Center for Immunology and Microbial Disease has a focus on microbial pathogenesis and immune defense, particularly as related to biothreat agents and emerging infections. Our faculty comprises a highly collaborative research group that received \$8.4M last year in NIH funding, ranking it within the top half of all Microbiology and Immunology programs in the country. The successful candidate(s) will receive attractive start-up packages and will have an opportunity to lead a focus group in new laboratory space, with access to all departmental core facilities including the Center's fully-staffed ABSL-3/BSL-3. We have established a close relationship with the New York State Department of Health Wadsworth Laboratories, providing a diverse environment that is rich in infectious disease expertise. Albany Medical College is located in a mid-sized city within the upstate New York Capital Region, and has easy access to Boston, New York City, and the Adirondack Mountains.

Applicants should send their curriculum vitae, a statement of research plans, and contact information for three references to:

Faculty Search Committee
Center for Immunology and Microbial Disease
Albany Medical College
47 New Scotland Avenue, MC-151
Albany, NY 12208

For further information about the Center, visit www.amc.edu/Academic/Research/imd.htm.

*An Equal Opportunity/Affirmative Action Employer.
Women and minorities are encouraged to apply.*



Director Program in Emerging Infectious Diseases

The Duke-NUS Graduate Medical School Singapore (Duke-NUS) is unique in bringing post-baccalaureate, research-intensive medical education to Asia, and represents a truly global partnership between two leading universities: Duke University (Duke) and National University of Singapore (NUS). Duke-NUS shares the modern Outram Campus with Singapore's largest hospital and several national research centers, and has created an academically based Signature Research Program in Emerging Infectious Diseases (EID) designed to serve as a national and international resource of excellence in emerging infectious diseases. The mission of the Program faculty is to conduct high-level basic and applied research, and to train graduate students, postdoctoral fellows, and clinician-scientists in the disciplines relevant to emerging infectious diseases.

We are seeking an individual with exceptional scientific credentials and leadership skills to head the EID Program. The position of Director will include full salary, a generous start-up and five years of annual research funding. The Director will be also provided with the space and resources necessary to recruit outstanding faculty members that will join a strong cadre of investigators that have already been recruited into the EID Program (see <http://www.duke-nus.edu.sg>).

Interested candidates should send a CV and the names of three references by 17 June 2011 to: **Search Committee on Emerging Infections, Duke-NUS Graduate Medical School, Singapore** by email to: director.eid@duke-nus.edu.sg

POSITIONS OPEN

POSTDOCTORAL POSITIONS

University of Maryland

Marlene and Stewart Greenebaum Cancer Center

Postdoctoral positions in the laboratory of **Dr. Ronald Gartenhaus** are available immediately to study the abnormal posttranscriptional gene regulation in lymphoma cells and how this contributes to lymphomagenesis; as well as the role of the MCT-1 oncogene in cellular translation and how it can be exploited for targeted lymphoma therapy (Dai et al, *Cancer Research*, 2009; Hagner et al, *Blood*, 2009; and Mazan-Mamczarz et al, *Blood*, 2011). Interested candidates must possess a recent Ph.D. with relevant experience in molecular biology. The laboratory is located in the Bressler Research Building with state-of-the-art facilities including core facilities for confocal microscopy, mass spectrometry, flow cytometry, bioinformatics, animal husbandry, etc. The University of Maryland Marlene and Stewart Greenebaum Cancer Center is an NCI-Designated Cancer Center.

Interested candidates should e-mail curriculum vitae, statement of research interests, and contact information for three references to **Ronald B. Gartenhaus, M.D.**, associate professor of medicine, the University of Maryland School of Medicine; e-mail: rgartenhaus@som.umaryland.edu.

BROOKLYN COLLEGE/CUNY

The Department of Chemistry seeks a **POSTDOCTORAL RESEARCH ASSOCIATE** for an NSF-funded project (Richard S. Magliozzo, PI) to study the heme enzyme catalase-peroxidase. Experience in metalloenzymes, spectroscopy, and enzymology preferred.

Salary commensurate with qualifications; complete benefits package.

Send letter and curriculum vitae to: **AVP Michael T. Hewitt, Human Resource Services, 2900 Bedford Avenue, Brooklyn, NY 11210-2889** or to e-mail: bcjobs@brooklyn.cuny.edu.

An Affirmative Action/Equal Opportunity/IRCA/ADA Employer.

ASSISTANT PROFESSOR CHEMISTRY/BIOCHEMISTRY—Tenure-track position at the College of Charleston, Charleston, South Carolina starting January 2012 or August 2012. The area of specialization is open but the successful candidate will teach the first semester Biochemistry course. Postdoctoral experience is highly desired. Teaching responsibilities will typically be nine contact hours per semester. The successful candidate must initiate and sustain a research program that includes undergraduates. Qualified women and minority candidates are encouraged to apply. Review of applications will begin April 8, 2011. An Equal Opportunity/Affirmative Action Employer. For full details, visit our website: <http://chemistry.cofc.edu/>.

NIH POSTDOCTORAL POSITION available to study zebrafish sensory neurobiology. The project will focus on characterizing the organization and function of lateral line system by combining electrophysiology with transgenic lines expressing fluorescent and photo-convertible proteins. Self-motivated, recent Ph.D.s with a strong publication record are encouraged to apply. Prior experience in patch clamp physiology, calcium imaging, or confocal imaging is preferred. Send curriculum vitae and contact information of three references to **Dr. James C. Liao** (website: http://www.whitney.ufl.edu/research_programs/liao.htm) at e-mail: jliao@whitney.ufl.edu.

POSITIONS OPEN

The newly established NIH-funded Center for Structure Determination of Membrane Proteins in Infectious Diseases (MPID) at Arizona State University is seeking to fill two positions at the **POSTDOCTORAL LEVEL** in protein biochemistry and X-ray crystallography. The mission of the center is the X-ray structure determination of membrane proteins involved in pathogenesis, and the development of new concepts and technologies to eliminate current bottlenecks in crystallization. Targets include viral, bacterial, and human membrane proteins. Requirements: Ph.D. in a related field such as Chemistry, Biochemistry, or Biophysics. Prior experience in biophysical characterization of membrane proteins or protein structure determination by X-ray crystallography is strongly preferred. Please submit cover letter, curriculum vitae, and contact information for three references as a single PDF file via e-mail attachment to: **Dr. Rebekka Wachter, Department of Chemistry and Biochemistry, Arizona State University, Tempe, AZ 85287-1604** (e-mail: rwachter@asu.edu). For additional information, please see websites: <http://sbkb.org/KB/centers.jsp?pageshow=8> and <http://cemilinks.asu.edu/mpid/>. Background check is required for employment. Arizona State University is an Equal Opportunity/Affirmative Action Employer committed to excellence through diversity. Women and minorities are encouraged to apply.

A **POSTDOCTORAL FELLOWSHIP** in Systems Neuroscience to study neural mechanisms of visual and cross-modal perception is available immediately at the Brain and Behavior Discovery Institute at Georgia Health Sciences University (GHSU). A variety of research projects is available in visual and cross-modal perception and rehabilitation of cognitive deficits. We use state-of-the-art research methods, including micro-electrode recordings in awake, behaving monkeys, fMRI in humans, human/monkey psychophysics, and computational modeling as well as neuropsychological assessment and rehabilitation of patients. Individuals with prior experience in at least one of these fields and the desire to gain expertise in one or more of the others are encouraged to apply. For more information about our research, visit the laboratory web page at website: <http://www.hegde.us>. Qualified candidates with a doctoral degree in neuroscience or an allied field should send a letter of interest and curriculum vitae to **Jay Hegde** at e-mail: jhegde@georgiahealth.edu. For additional information about institutional requirements and to apply, visit GHSU's employment page (website: <http://www.georgiahealth.edu/jobs/external.html>) and apply for position #9639. GHSU is an Affirmative Action/Equal Employment Opportunity/Equal Access/ADA Employer.

Mount Sinai School of Medicine has **POSTDOCTORAL POSITIONS** available through its NCI funded training program in cancer biology. Areas of interest include translational aspects of cellular signaling, developmental biology, cell cycle, or molecular epidemiology. These positions are open to U.S. citizens or permanent residents with an M.D. or Ph.D. with experience in molecular/cellular biology. Salary will follow NIH guidelines and depend on experience. For a list of training faculty, please see website: <http://fusion.mssm.edu/training/facilist2.cfm?sec=8>. Highly motivated candidates are encouraged to send or e-mail an application including a cover letter, curriculum vitae, and contact information of three references to: **Postdoctoral Training in Cancer Biology, MSSM, One Gustave L. Levy Place, Box 1130, New York, NY 10029**. E-mail: cancer.biology@mssm.edu.

POSTDOCTORAL POSITIONS in Synthetic Chemistry

The International Institute of Nano and Molecular Medicine of the University of Missouri is seeking candidates to fill grant-funded postdoctoral research positions in Synthetic Organic Chemistry. The Institute houses world-class laboratories and instrumentation described on our website: <http://nanomed.missouri.edu>. Compensation will be determined by past experience and training. Electronically submit curriculum vitae and a description of research to e-mail: nanomed@missouri.edu. MU is an Equal Employment Opportunity/Affirmative Action/ADA employer and encourages applications from women and minorities.

FACULTY POSITION



Cornell Laboratory for Accelerator-based Sciences and Education (CLASSE)

SENIOR RESEARCH ASSOCIATE

Cornell Laboratory for Accelerator-based Sciences and Education

The Cornell Laboratory for Accelerator-based Sciences and Education (CLASSE) seeks an individual to take a lead role in the management and development of major accelerator systems. The specific research and development activities will be in one of the accelerator-associated fields that are part of the mission of CLASSE (X-ray science, accelerator science, or elementary particle physics). These activities will be determined in consultation with the CLASSE Director, to whom this position reports. Duties will also include overseeing accelerator radiation protection and safety systems; performing radiation shielding calculations using Monte Carlo codes such as MARS, FLUKA, EGS5, or MCNPX; calculating radioactivity induced in materials by stray radiation fields around electron accelerators; assessing radiation damage in electronics; making measurements of photon and neutron spectra over a wide energy range; and reviewing and approving safety analysis documents for laboratory operations, working with staff at all levels to analyze and set procedures for unusual or new safety issues.

Requirements: A Ph.D. in one of the physical sciences or engineering, and several years of experience in experimental high-energy accelerator physics or nuclear science and engineering. Must be familiar with biological effects from natural and man-made sources of environmental radioactivity; federal, state, and local laws governing radiation; photon and neutron dosimetry; and occupational, chemical, biological, and laser safety. A high level of people skills and mature judgment are essential.

Please send a cover letter, including curriculum vitae and a publications list to **Dr. Maury Tigner**, Newman Laboratory, Cornell University, Ithaca, NY 14853, and arrange for three letters of recommendation to be sent. Correspondence may be directed to e-mail: search_classe@cornell.edu. Cornell University is an Equal Opportunity/Affirmative Action Educator and Employer.

POSTDOCTORAL POSITION

POSTDOCTORAL RESEARCH SCIENTIST

Columbia University
New York, NY

The Department of Anesthesiology at Columbia University Medical Center, College of Physicians and Surgeons, seeks Postdoctoral Research Scientists to work in the laboratory of **Joachim Scholz, M.D.** Research in the laboratory focuses on the causes and consequences of peripheral neuropathy, and on mechanisms contributing to the development of chronic inflammatory or neuropathic pain. The laboratory is using in vivo and in vitro models of disease and pursues a multidisciplinary approach that includes molecular biology, stem cell technology, imaging, neurosterology, and behavioral studies.

Competitive candidates must have a Ph.D. or M.D.-Ph.D. degree and strong publication record in neuro-science, immunology, or stem cell biology. One of the positions is designed for an electrophysiologist with profound expertise in patch-clamp recordings from brain or spinal cord slice preparations. Website: <http://www.cumc.columbia.edu/dept/anesthesiology/research/neuroscience.html>.

Applicants should send a cover letter stating their research interests, curriculum vitae, and contact information for three references to: **Joachim Scholz, MD**, at e-mail: js3932@columbia.edu. Columbia University is an Equal Opportunity/Affirmative Action Employer.



Nontraditional Careers: Opportunities Away From the Bench Webinar

Want to learn more about exciting and rewarding careers outside of academic/industrial research? View a roundtable discussion that looks at the various career options open to scientists and strategies you can use to pursue a nonresearch career.

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HOCHSCHULE
AACHEN

JARA | Jülich Aachen
Research
Alliance

JÜLICH
FORSCHUNGSZENTRUM

Focusing Expertise – Shaping the Future: The Jülich Aachen Research Alliance (JARA) is an innovative cooperation model between RWTH Aachen University and Forschungszentrum Jülich. This Alliance brings together an internationally respected university of technology and one of the leading national research centres in Europe. The following position for a director is for the JARA-Energy section.

The Institute of Energy and Climate Research (IEK) at Forschungszentrum Jülich conducts research on pioneering technologies for energy conversion and storage and examines the consequences of energy use and the related emissions.

Forschungszentrum Jülich, in a joint procedure with RWTH Aachen University, is seeking at the earliest possible starting date a scientist as

DIRECTOR

for the newly founded institute division

„Principles of Electrochemistry“

at the Institute of Energy and Climate Research (IEK)

In accordance with the „Jülich Model“, the successful applicant will also be appointed professor (grade W3) of „materials and processes for electrochemical energy storage systems and converters“ in the Department of Chemistry in the Faculty of Mathematics, Computer Science and Natural Sciences at RWTH Aachen University.

The professorship is intended to intensify research on electrochemical energy technologies within Forschungszentrum Jülich as well as research with RWTH Aachen University in the field of fundamental electrochemistry. The main areas of research will comprise in particular experimental investigations on electrochemical and catalytic properties and processes, the primary synthesis of new ion-conducting and electrochemically active materials, as well as the modelling of electrochemically relevant properties and mechanisms for energy technologies. Furthermore, research work will contribute decisively to understanding of and model development for degradation in electrochemical energy converters and storage systems. Intensive cooperation with other areas of Forschungszentrum Jülich in the fields of batteries, fuel cells, gas separation membranes and electrolyzers is therefore an integral part of this position.

The successful candidate will have proven scientific excellence in the following fields:

- new material classes for electrochemical applications
- experimental characterization of charge transport and interface reactions
- theoretical model concepts as a basis for the targeted development of new materials with tailor-made electronic, ionic and catalytic properties

The successful applicant will benefit from the excellent infrastructure of a national research centre and a high degree of freedom in establishing and expanding a new institute division.

Prerequisites for the position are a PhD and postdoc qualification or comparable scientific achievements. The ability and willingness to participate in university teaching as well as the successful acquisition of third-party funds are also a requirement.

Applications from women are particularly welcome. The implementation of equal opportunities is a cornerstone of staff policy at Forschungszentrum Jülich and RWTH Aachen University for which both institutions have received the „TOTAL E-QUALITY“ award. Applications from women will be given preference in the case of equal suitability, qualifications and experience, unless special reasons concerning the person of a male candidate outweigh these considerations. Attention is drawn to Art. 8, para. 1, of the Equal Opportunities Act of the Federal State of North Rhine-Westphalia (LGG).

Applications from suitable candidates with disabilities are explicitly encouraged. This also holds for those with similar incapacities in the sense of Art. 2 SGB IX. RWTH Aachen University has been named as a „disability-friendly“ employer for its commitment to training and employing disabled people.

Applications comprising a curriculum vitae, list of publications and a short summary of past and planned scientific projects should be sent, **preferably by email**, by 16 May 2011 to

Board of Directors
Forschungszentrum Jülich GmbH
52425 Jülich
Germany
berufungen@fz-juelich.de

Further information at
www.fz-juelich.de
www.chemie.rwth-aachen.de | www.jara.org



PROGRAM OFFICER HUMAN SYSTEMS INTEGRATION

(Cognitive Scientist, Computer Scientist, or General Engineer)

The Office of Naval Research is seeking a qualified individual to manage sponsored basic/applied research, and advanced technology development programs and projects in the broad areas of human systems integration (HSI), psychology, human factors science and technology, and cognitive science. The sponsored efforts are conducted principally at U.S. universities and industry or Federal laboratories. This is a Civil Service position at the NP-IV level (\$105,211 - \$155,500) depending on individual qualifications.

The position requires knowledge and experience in the fundamental theories, concepts, and current-state-of-the-art research and/or technology development in the broad areas of human systems integration (HSI), psychology, human factors science and technology, and cognitive science, including but not limited to, human decision-making, HSI engineering design, intelligent systems, training, performance support, human-computer interaction, and HSI modeling and simulation.

For information on qualifications and how to apply, see the links to our Job Announcements at <http://www.onr.navy.mil/en/career-job-opportunity/job-listing.aspx>. Applications must be submitted by close of business or postmarked as of the closing date noted in the job announcement.

U.S. CITIZENSHIP REQUIRED • AN EQUAL OPPORTUNITY EMPLOYER



THE HONG KONG UNIVERSITY OF SCIENCE AND TECHNOLOGY

School of Science Head of the Division of Life Science

The School of Science of the Hong Kong University of Science and Technology (HKUST) is currently looking for an outstanding academic to lead the newly established Division of Life Science as its Founding Head. Opened in October 1991, HKUST is an English medium research-intensive university dedicated to the advancement of learning and scholarship, with special emphasis on postgraduate education, and close collaboration with business and industry. Despite its relatively short history, it has rapidly grown into a major research university in Hong Kong and the world.

To raise Life Science teaching and research at HKUST to a new level of excellence, the Division of Life Science was established through the re-organization of the Departments of Biochemistry and Biology. The Division currently has 29 faculty members with a range of expertise (cell signaling, cancer biology, cell and developmental biology, neuroscience, structural biology, marine and environmental science, and biotechnology), and is expected to grow substantially in terms of academic staff, programs, resources and facilities. It will also create synergies in research and scholarship by enhancing collaborations within the Division as well as interdisciplinary research with other disciplines.

Reporting to the Dean of Science, the Head of the Division is expected to lead the Division, formulate policies and be responsible for the Division's academic advancement in both teaching and research, recruitment and resource allocation. He/She is also expected to devise strategies to promote and facilitate collaborative and interdisciplinary research with other disciplines.

Applicants should be academics with an excellent record of scholarship eligible for a senior appointment at Professor level in the discipline of life sciences; experience in leading multi-disciplinary collaborative research programs; proven leadership and managerial effectiveness at an appropriate level in higher education institutions; an appreciation of the breadth and depth of research and educational activities in life sciences; and outstanding communication and interpersonal skills.

Professorial appointment with tenure will be made to the successful candidate. Salary is highly competitive and will be commensurate with qualifications and experience. Generous fringe benefits will also be provided.

Application package, including a curriculum vitae, a vision statement as well as the names, addresses, phone numbers and email addresses of at least three referees should be sent to: **Office of the Dean of Science (Re: Head of the Division of Life Science), The Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong** (or by fax: (852) 2358 1464; email: dissearch@ust.hk). Review of applications will begin immediately and will continue until the position is filled.

For further information about HKUST, the School of Science and the Division of Life Science, please visit the following websites:

HKUST - <http://www.ust.hk>
School of Science - <http://science.ust.hk>
Division of Life Science - <http://life-sci.ust.hk>



ASSISTANT/ASSOCIATE PROFESSOR

Department of Molecular Medicine and USF Health Byrd Alzheimer's Institute

The USF Health Byrd Alzheimer's Institute and Department of Molecular Medicine at the USF Health College of Medicine are partnering to identify a candidate for a tenure track position at the rank of Assistant or Associate Professor. A successful applicant will employ structural, molecular and/or computational approaches to develop a mechanism-based drug discovery program related to neurodegenerative disease. The USF Health Byrd Alzheimer's Institute is a unique enterprise that allows investigators to participate in basic and clinical studies directly related to Alzheimer's disease and other dementias in one building. The Institute is the largest of its kind in the world. The Department of Molecular Medicine and USF Health Byrd Alzheimer's Institute are located in a vibrant and expanding USF Health Complex on the main campus of the University of South Florida that includes the Colleges of Medicine, Nursing, and Public Health, and the H. Lee Moffitt Comprehensive Cancer Center and Research Institute. USF is a nationally recognized research campus with extramural grants and contract activity in excess of \$350 million annually and is home to several Centers for Excellence in Biomedical Research and state-of-the-art facilities. Over the past 3 years, the state of Florida committed an additional \$450 million to the existing medical research budget, and as a result, USF is now part of a collaborative research corridor running through the heart of the sunshine state.

Successful candidates for this position are expected to develop and maintain a competitively funded research program, and to participate in medical and graduate education. To support these expectations, the department offers strong institutional salary support, competitive start-up packages, excellent core facilities, and a collegial atmosphere to create an environment that promotes success. Academic rank and salary are commensurate with qualifications and experience. The successful candidate must have a doctorate in a biomedical sciences discipline with at least two years postdoctoral experience. Appointment at the level of Associate Professor requires a minimum of five years experience at the Assistant Professor level, a nationally-recognized and federally funded research program and experience mentoring graduate students.

Please send a cover letter, curriculum vitae, research plan, statement of teaching philosophy, and arrange three reference letters to be sent by e-mail (with attached PDFs) to Ms. Maxine Roth at mroth@health.usf.edu. Review of applicants will begin immediately and continue until the position is filled. **Dr. Chad Dickey, Search Committee Chair, University of South Florida-Health, 12901 Bruce B. Downs Blvd. MDC7, Tampa, Florida 33612-4799**

USF Health is committed to increasing its diversity and will give individual consideration to qualified applicants for this position with experience in ethnically diverse settings, who possess varied language skills, or who have a record of research that support diverse communities or teaching a diverse student population. The University of South Florida is an EO/AA Employer. For disability accommodations, contact Maxine Roth at (813) 396-0746 a minimum of five working days in advance. According to FL law, applications and meetings regarding them are open to the public.



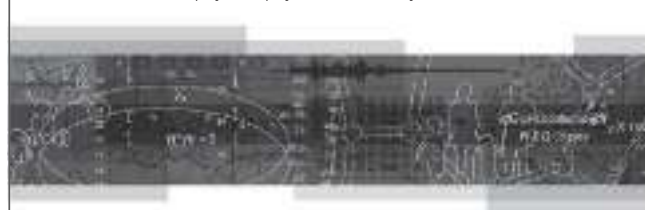
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CHAIR, DEPARTMENT OF HEMATOLOGY

St. Jude Children's Research Hospital is seeking a Chair for the Department of Hematology on the rank of full member. A commensurate appointment at the full professor rank at the University of Tennessee is included. The position requires visionary thinking to build on St. Jude's foundation of excellence within an interdisciplinary environment. The successful candidate must have an M.D. or M.D./Ph.D. with a strong research interest, proven extramural funding, and solid clinical background. The candidate must possess strong administrative and communication skills, a commitment to education and training, and strong leadership capabilities.

St. Jude's hematologists are an integral part of a multidisciplinary team that includes oncologists, bone marrow transplant therapists, radiation therapists, pathologists, radiologists, infectious disease specialists, basic scientists and biostatisticians. The Hematology department has a substantial clinical program, including one of the largest pediatric sickle cell disease populations in the U.S. and an established NIH-funded research program with ample opportunities in basic and clinical research. There are twelve faculty members equally distributed between clinical and basic science. The department supports a strong translational research program in gene therapy as well as diverse research and clinical programs in molecular control of blood cell development and regulation, sickle cell disease, hemophilia, thrombophilia, marrow failures and platelet disorders. Further opportunities are available for collaboration and integration with a broad array of programs in the LeBonheur Children's Hospital and the University of Tennessee Memphis.

St. Jude is a leading institution in pediatric hematology-oncology, and the future chair is expected to be a leader in his/her field as well as an active member of the St. Jude leadership. St. Jude ranks number 1 in pediatric cancer hospitals and is on the *Fortune* magazine's list of 100 best places to work. All patients accepted for treatment at St. Jude are treated without regard to the family's ability to pay.

The campus is located on 70 acres in downtown Memphis on the banks of the Mississippi River. Memphis offers a low-cost-of-living as well as numerous cultural attractions, including historic sites like Beale Street, two symphony orchestras, opera and ballet companies, great music (home of Elvis Presley, Graceland, and Sun Studios), many museums, and a dynamic live theatre community. Memphis is home to the Memphis Grizzlies Basketball Team, hosts the St. Jude PGA Golf Tournament, and is a stop on the Kroger St. Jude Tennis Tour.

Interested individuals are asked to submit a cover letter and C.V. to:

Joseph H. Laver, M.D.
Clinical Director
St. Jude Children's Research Hospital
262 Danny Thomas Place
Memphis, TN 38105
E-mail: joseph.laver@stjude.org

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POSTDOC OPPORTUNITIES



Postdoctoral Positions in Neuroimmunology Barrow Neurological Institute Phoenix, Arizona, USA

NIH- and MDA-funded postdoctoral positions are available in the Neuroimmunology Laboratory to study the innate immunity and inflammation in neurological diseases.

Sound knowledge and hands on experience in one or more of the following areas are preferred: human immunology, cell signaling, neural stem cells, creation of (CNS)-transgenic mice, murine models of disease. For more information, please visit: thebarrow.org/Research/Neuroimmunology/205490.

To apply, submit a curriculum vitae, a brief statement of your qualifications, research interest and future career plans, as well as names and contact information of three references to **Dr. Ruolan Liu: Ruolan.liu@chw.edu**.

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Chair in Structural Biology Faculty Appointment in Biochemistry and Molecular Biology

The Medical University of South Carolina seeks to recruit an outstanding scientist as part of the South Carolina Center of Economic Excellence in Drug Discovery and Endowed Chair Program in Structural Biology. Applications are sought from investigators with interests in any area of structural biology, ranging from the high-resolution techniques of X-ray crystallography and NMR spectroscopy to lower resolution techniques such as scanning probe microscopy, low-angle scattering, single molecule fluorescence, cryo-electron microscopy or computational methods. Although any area of biomedical research will be considered, we are particularly interested in candidates whose research program complements existing strengths in cancer biology, lipid signaling and lipid-based therapeutics, RNA structure and metabolism and DNA replication. Candidates must have demonstrated ability to secure peer-reviewed research funding and will be expected to lead broader programmatic development on campus in his/her area of work. The appointment will be at the Associate or Full Professor level, commensurate with experience.

Extramural research funding to MUSC exceeds \$234 million, reflecting tremendous growth in the research enterprise, including a National Cancer Institute-designated cancer center and an NIH Clinical and Translational Science Award. Two new research buildings dedicated to interdisciplinary work in Drug Discovery, Bioengineering and Cancer Genomics will open this summer. An NMR facility in the Drug Discovery Building will house 400 and 600 MHz magnets and a new 850 MHz spectrometer which is slated for installation in October 2011. As a founding member of SER-CAT, MUSC has guaranteed access to two synchrotron beam lines at Sector 22 of the Advanced Photon Source, Argonne National Lab. in Illinois.

This is an exceptional opportunity for a talented, experienced and committed individual to exert a major impact on the next phase of development and growth of the research and translational sciences programs at MUSC.

Located on the Atlantic coast in South Carolina, Charleston boasts one of the nation's most historic downtown areas, beautiful beaches and international cultural events such as the Spoleto Festival USA.

To apply, interested candidates should submit their CV, a summary of future research plans and names of three references to: **Eleanor Spicer, Ph.D., Department of Biochemistry and Molecular Biology, 173 Ashley Avenue, Charleston, SC 29425**, or via e-mail to fulghumk@musc.edu.

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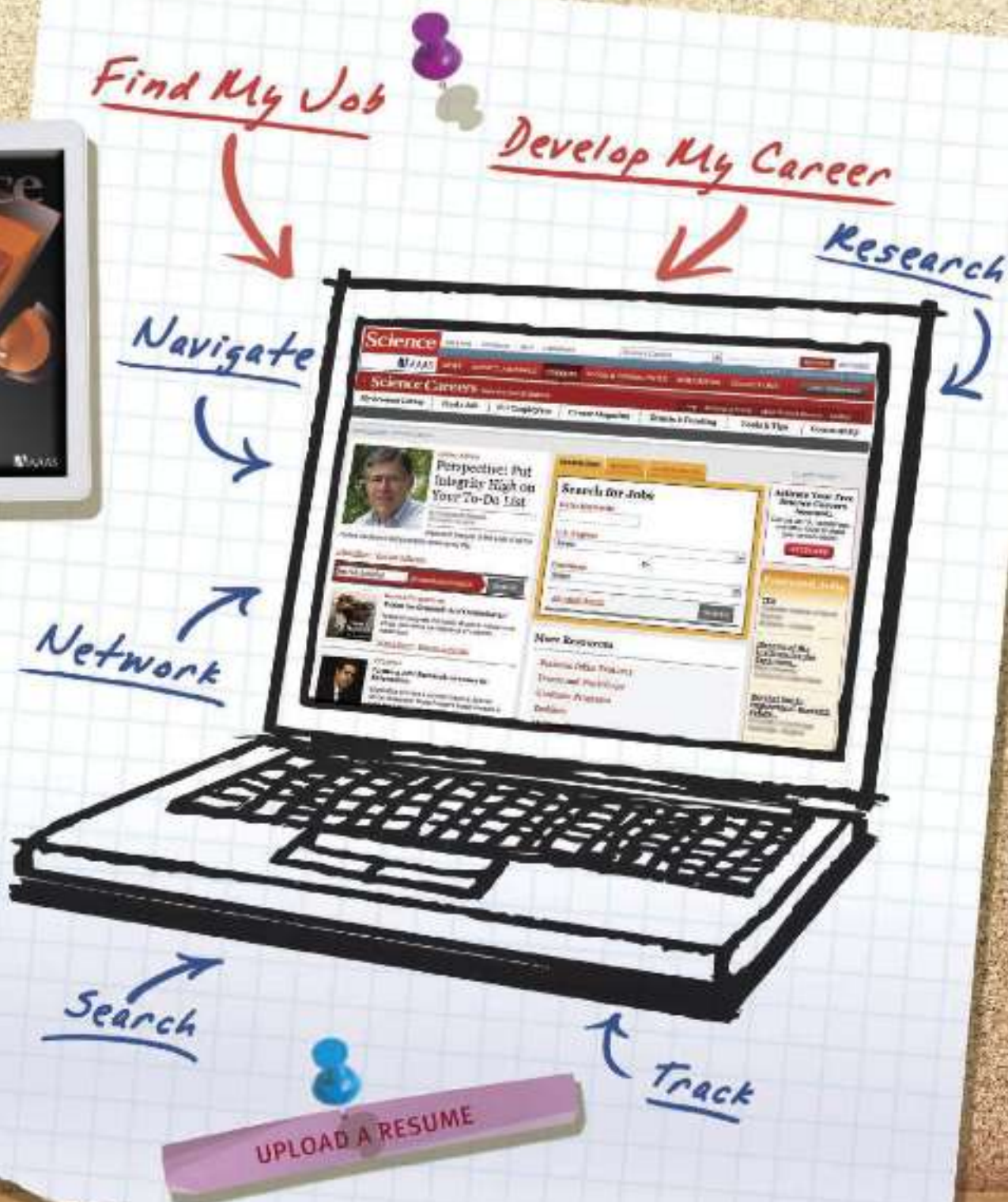
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Assistant Professor Food and Bioproduct Sciences

The Department of Food and Bioproduct Sciences at the University of Saskatchewan invites applications for a tenure track position at the Assistant Professor level. Candidates are expected to contribute to the nanotechnology research focus area. A Ph.D. in Food Science or a related discipline with a distinguished academic record is required. Relevant post-doctoral research and teaching experience would be an asset. Responsibilities include teaching undergraduate and graduate courses and leading an externally funded, scholarly research program. The successful candidate will be able to do cross-cutting (i.e. food and bioproduct) research within the department and collaborate effectively with researchers within and beyond the department.

More information about the University of Saskatchewan, College of Agriculture and Bioresources, and the Department of Food and Bioproduct Sciences can be found at <http://www.agbio.usask.ca>.

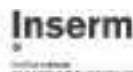
Applicants should submit a curriculum vitae, a concise teaching and research vision statement, and the complete contact information for three referees. Application deadline is **May 2, 2011**. Applications should be sent to: **Dr. Nicholas H. Low, Professor and Head, Department of Food and Bioproduct Sciences, University of Saskatchewan, 51 Campus Drive, Saskatoon, SK, Canada, S7N 5A8. Email: nicholas.low@usask.ca**.



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The Paris-Cardiovascular Research Center (PARCC, INSERM U970) is recruiting a new group.

The Paris-Cardiovascular Research Center (PARCC, INSERM U970) is recruiting a new group.

The PARCC (<http://parcc.inserm.fr/>) is a research center located at the Georges Pompidou European Hospital (HEGP), Paris, funded by INSERM and University Paris-Descartes. The PARCC is involved in projects that span from basic molecular and cellular biology to integrated physiology and pathophysiology, pharmacology, biomarkers, genetics and epidemiology, with the goal of understanding the development and complications of major cardio-vascular disease, including atherosclerosis, hypertension, heart failure and sudden cardiac death. Translational research is an integral part of our activities, in close connection with clinical laboratories and departments of the HEGP. The PARCC is comprised of 11 research groups, representing a total of approx. 200 staff and students, and provides a dynamic and synergistic research environment.

The PARCC will make available 50-60 m² of equipped laboratory space. The new group will have access to state-of-the-art technological core facilities within the center, including flow cytometry and advanced molecular imaging platforms.

Application deadline: **June 15, 2011**

Please include: CV, list of publications, group composition, short description of previous achievements, proposed research program (no longer than 10 pages), 2 letters of recommendation.

Audition and seminar of short-listed candidates: September 2011.

Results of the search: October 2011

Contact: Alain Tedgui (alain.tedgui@inserm.fr)

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PRIZES



The Martha T. Muse Prize for Science and Policy in Antarctica 2011

(Nominations close 1 May 2011)

The Martha T. Muse Prize is an annual \$100,000 unrestricted award to an individual who has demonstrated excellence in Antarctic science and/or policy and who shows clear potential for sustained major and significant contributions that will enhance the understanding of Antarctica. The Prize is inspired by Martha T. Muse's passion for Antarctica and is intended to be a legacy of the International Polar Year 2007–2008. The Prize is awarded by the Tinker Foundation and administered by the Scientific Committee on Antarctic Research (SCAR).

The prize-winner can be from any country and work in any field of Antarctic (including Southern Ocean) science and/or policy. The goal is to recognise the important work being done by the individual and to call attention to the significance of understanding Antarctica in a time of change.

Further details, including the process of online nomination are available at www.museprize.org.

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Heidelberg-Kyoto Joint Symposium Crossing Boundaries: Stem Cells, Materials, and Mesoscopic Sciences

- Heidelberg University/Universitätsklinikum, SFB 873
- Kyoto University Institute for Integrated Cell-Material Sciences (iCeMS)

Call for Posters

Thursday 21 July

- Keynote 1: François Brochard-Wyart, Institut Curie
"Crossing boundaries from mesoscopic physics to biological functions: Wetting transitions of living drops"
- Keynote 2: Austin Smith, Wellcome Trust Centre for Stem Cell Research
"Ground rules for pluripotent stem cells"

Friday 22 July

- Session 1: Evolution of Stem Cell Systems
- Session 2: Pluripotency and Reprogramming
- Session 3: Niche and Polarity
- Session 4: Cell-Material Integration

Saturday 23 July

- Session 5: Meso-Bio Single-Molecule Imaging
- Session 6: Chemical Biology & Nano/Meso/Micro-Engineering

Speakers

- Wakazu Agata.....(Kyoto Univ Graduate School of Science)
- Reinut Aigner.....(Heidelberg University & DKFZ)
- Michael Boudos.....(Heidelberg University & DKFZ)
- François Brochard-Wyart.....(Institut Curie)
- Yong Chen.....(Kyoto University, CeMS & ENS)
- Denis Choquet.....(University of Bordeaux)
- Bruce Edgar.....(Heidelberg University ZMBH)
- Shuhei Furukawa.....(Kyoto University, CeMS)
- Elke Günther.....(University of Tübingen)
- Yoshio Harada.....(Kyoto University, CeMS)
- John Heuser.....(Kyoto Univ, CeMS & Washington Univ)
- Takanori Hiragi.....(Kyoto Univ, CeMS & EMBL Heidelberg)
- Anthony D Ho.....(Heidelberg University)
- Thomas Holstein.....(Heidelberg University)
- Sachiko Kikugawa.....(Kyoto University, CeMS)
- Jörg Lahann.....(KIT & Michigan University)
- Jan Lohmann.....(Heidelberg University)
- Russel Morris.....(University of St Andrews)
- Tatsuya Murakami.....(Kyoto University, CeMS)
- Norio Nawatsugi.....(Kyoto University, CeMS)
- Reisuke Okita.....(Kyoto University CIRA)
- Michael Saitou.....(Kyoto Univ Graduate School of Medicine)
- Hans R Schöler.....(MPI Münster)
- Austin Smith.....(Wellcome Trust Centre for Stem Cell Resrch)
- Yoshio Suda.....(Keio University School of Medicine)
- Yoshinori Sugiyama.....(Kyoto University, CeMS)
- Kenichi Suzuki.....(Kyoto University, CeMS)
- Motomu Tanaka.....(Heidelberg University)
- Andreas Trumpf.....(German Cancer Research Center, DKFZ)
- Motonari Uesugi.....(Kyoto University, CeMS)
- Jochen Ueber.....(Heidelberg University)
- Johann Wenzel.....(Heidelberg University)

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Universitätsklinikum Heidelberg

POSITIONS OPEN



VISITING ASSISTANT PROFESSOR OF Biology

Department of Biology

The Biology Department at the University of the South invites applications for a Visiting Assistant Professor for the 2011/2012 academic year. The successful candidate will teach two sections of the Department's introductory field investigations in biology class and one section of biology for nonmajors (tree contact hours teaching time per section), with at least two sections taught during the Spring 2012 semester. Candidates should be enthusiastic about working in the context of the liberal arts tradition in education.

The University of the South, familiarly known as Sewanee, consists of a highly selective, 1,425-student College of Arts and Sciences and a 70-student School of Theology. Located on a 13,000-acre campus on Tennessee's Cumberland Plateau, it is an institution of the Episcopal Church that welcomes individuals of all backgrounds.

Review of applications will begin April 4, 2011, and continue until the position is filled.

Eligibility for employment is contingent upon successful completion of a preemployment screening.

Send a letter of application, curriculum vitae, statements of teaching and research interests, transcripts, and three letters of reference to:

Tracy Hall
Faculty Hiring Specialist
The University of the South
735 University Avenue
Sewanee, TN 37383-1000

Electronic submission is preferred e-mail: thall@sewanee.edu.

The University of the South is an Equal Opportunity Employer. Women and minorities are encouraged to apply.

Department of Earth and Marine Sciences

Dowling College seeks to fill a full-time, tenure-track **GENERAL PHYSICAL SCIENTIST POSITION** in the Department of Earth and Marine Sciences. A Ph.D. in a related field from an accredited college or university is required at time of appointment. Experience or ability to teach introductory earth sciences courses, field sampling and data collection techniques, and undergraduate and graduate courses in earth and marine sciences is required. The candidate must have a demonstrated interest and active involvement in field research. We are seeking to fill this position at the **ASSISTANT PROFESSOR LEVEL**; however, rank and salary are dependent upon the appointee's qualifications and experience.

Qualified candidates should send a letter of application, resume, transcripts (unofficial copies are sufficient for initial review), a brief statement of research interests, a brief statement of teaching philosophy, and three letters of recommendation to: **Dr. John T. Tanacredi, Chair of the Search Committee, Department of Earth and Marine Sciences,**

Dowling College, Kramer Science Center, 150 Idle Hour Boulevard, Oakdale, New York 11769, e-mail: tanacrej@dowling.edu.

A copy of your letter of application and curriculum vitae should also be sent to: **Dowling College, Human Resources Department, Kramer Science Center, 150 Idle Hour Boulevard, Oakdale, New York 11769, e-mail: hrrsumes@dowling.edu or fax: 631-244-3006.**

Dowling College is an Equal Opportunity/Affirmative Action Employer.

POSTDOCTORAL POSITION is available for a Ph.D. with experience in synaptic plasticity, electrophysiology, and synaptic activity. The research project is to elucidate the mechanisms of neurodegeneration in Aging and Alzheimer's disease. Candidate should have a strong publication related to the neurophysiology, synaptic plasticity, and synaptic vesicle. The position is available at University of Kansas. Send your resume to **Dr. Shirley Yan (e-mail: shidu@ku.edu).**

POSITIONS OPEN



MOLECULAR PHARMACEUTICS IMAGING FACULTY POSITION

The Division of Molecular Pharmaceutics (MOPH), Eshelman School of Pharmacy (ESOP) and the Biomedical Research Imaging Center (BRIC) of the University of North Carolina at Chapel Hill are jointly seeking outstanding candidates for a tenure-track faculty position with research and teaching expertise in molecular imaging. The 12-month appointment will be at the **ASSISTANT PROFESSOR** level. MOPH will be the academic home for the successful candidate. The new faculty member's research and teaching responsibilities should complement and further strengthen the existing programs in the Division (drug/gene delivery). Expertise in all imaging modalities is appropriate, but preference will be for PET or SPECT imaging. Exceptional opportunities for multimodality imaging research in animal models as well as close interactions between researchers in nanotechnology, chemistry, physics, and material sciences to those in cancer biology and clinical science exist at UNC. Qualified candidates should have a doctoral degree in chemistry, pharmaceutical sciences, or an equivalent field. The successful candidate is expected to establish an independent research program and to effectively contribute to the missions of both the ESOP and BRIC, and to secure extramural grant funding. Candidates should apply at website: jobs.unc.edu/2500964 and attach the following materials as a single PDF file: a letter of interest addressed to **Dr. Michael Jay** (Search Committee Chair; UNC Eshelman School of Pharmacy; UNC Chapel Hill; Chapel Hill, North Carolina 27599), a complete curriculum vitae, a detailed research statement, and names and full contact information for four references. Review of applications will begin immediately and the position will remain open until the appointment is made. *Women and members of minority groups are especially encouraged to apply. The University of North Carolina at Chapel Hill is an Equal Opportunity Employer.*

DIRECTOR

Eastman Institute for Oral Health

The University of Rochester Medical Center (URMC) is seeking a Director of the Eastman Institute for Oral Health, a division of URMC. The Institute is nationally recognized for excellence in patient care, community service education, and research. It contains more than 180 full- and part-time faculty members, 90 residents and fellows, and over 200 associated support staff. Community-oriented, multidisciplinary, general and specialty, oral health clinical services are provided with over 145,000 patient visits per year in multiple sites, on the campus and in the community. Postdoctoral training includes advanced clinical education in general dentistry (AEGD), general practice residency (GPR); oral and maxillofacial surgery; orthodontics and dentofacial orthopedics; pediatric dentistry; periodontology; and prosthodontics and a fellowship in oro-facial pain. The Institute has a robust research program that consists of extramurally funded integrated programs in basic, translational, and clinical research. The URMC was ranked fourth among dental institutions in NIDCR funding in 2009.

The Committee is searching for a candidate with broad experience in the field of Dentistry with outstanding leadership skills. The candidate should be committed to excellence in patient care, research, and education, and have the ability to foster programmatic growth through innovation, collaboration, and technology development. He/she should have the academic qualifications for appointment as Chair and Professor of Dentistry. Inquiries should be addressed to: **Mark Taubman, M.D., Dean, University of Rochester School of Medicine and Dentistry, c/o Sharon Kubiak, Manager, Candidate Administration Services, University of Rochester Medical Center, 601 Elmwood Avenue, Box 706, Rochester, NY 14642; or e-mail: sharon_kubiak@urmc.rochester.edu.** *The University of Rochester has a strong commitment to principles of diversity and, in that spirit, actively encourages applications from groups underrepresented in higher education*

POSITIONS OPEN

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Contact the Admissions Office, telephone: 800-824-5526 at: The New England College of Optometry, 424 Beacon Street, Boston, MA 02115. Additional information at website: <http://www.neco.edu>, or by e-mail: admissions@neco.edu.

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Call for Symposium Proposals

Flattening the World: Building the 21st Century Global Knowledge Society

We live in a time when collaborations between countries and continents have never been easier, at least from a technical standpoint. A stunning example is the Large Hadron Collider, which is being used by a multinational group of physicists to understand the fundamental building blocks and laws of nature, from subatomic to cosmic. Stores of information and knowledge can be accessed from anywhere by anyone. Remote sensing technology enables the detailed observation of virtually every aspect of our planet's surface, subsurface, and climate. Technology and the Internet are transforming education. Learning is, in principle, available to everyone everywhere.

The 21st century is shaping up to be a challenging one. The issues that face us are many: climate change, energy, agriculture, health, water, biodiversity and ecosystems, population growth, and economic development. The 2012 program will focus on the complex challenges of the 21st century that are both global in their scope and profoundly interconnected as well as ways to tackle them on a global scale through international, multidisciplinary efforts.

Symposium proposals for the 2012 meeting are now being solicited. To submit a proposal, visit www.aaas.org/meetings. The deadline for submission is Tuesday, 26 April 2011.

Call for Poster Submissions

Student Poster Competition

Open to college undergraduate and graduate students only

The competition recognizes the individual efforts of students who are actively working toward a college-level degree. Winners in each category receive a cash award and framed certificate, and are listed in *Science*.

General Poster Session

Open to postdocs and professionals

This session provides an opportunity for postdocs and professionals to present their research to the broad community of scientists attending the AAAS Annual Meeting.

Information about the call for poster submissions for the 2012 Annual Meeting will be available at aaas.org/meetings on 12 May 2011.

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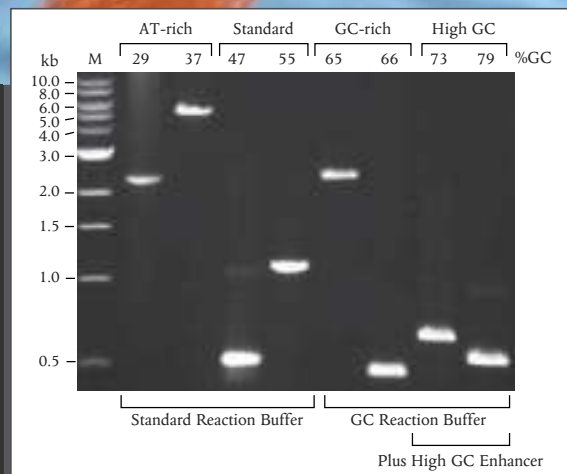
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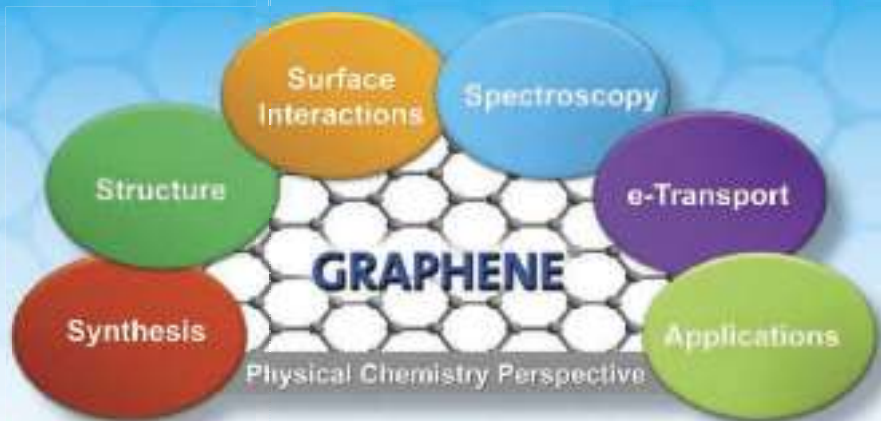
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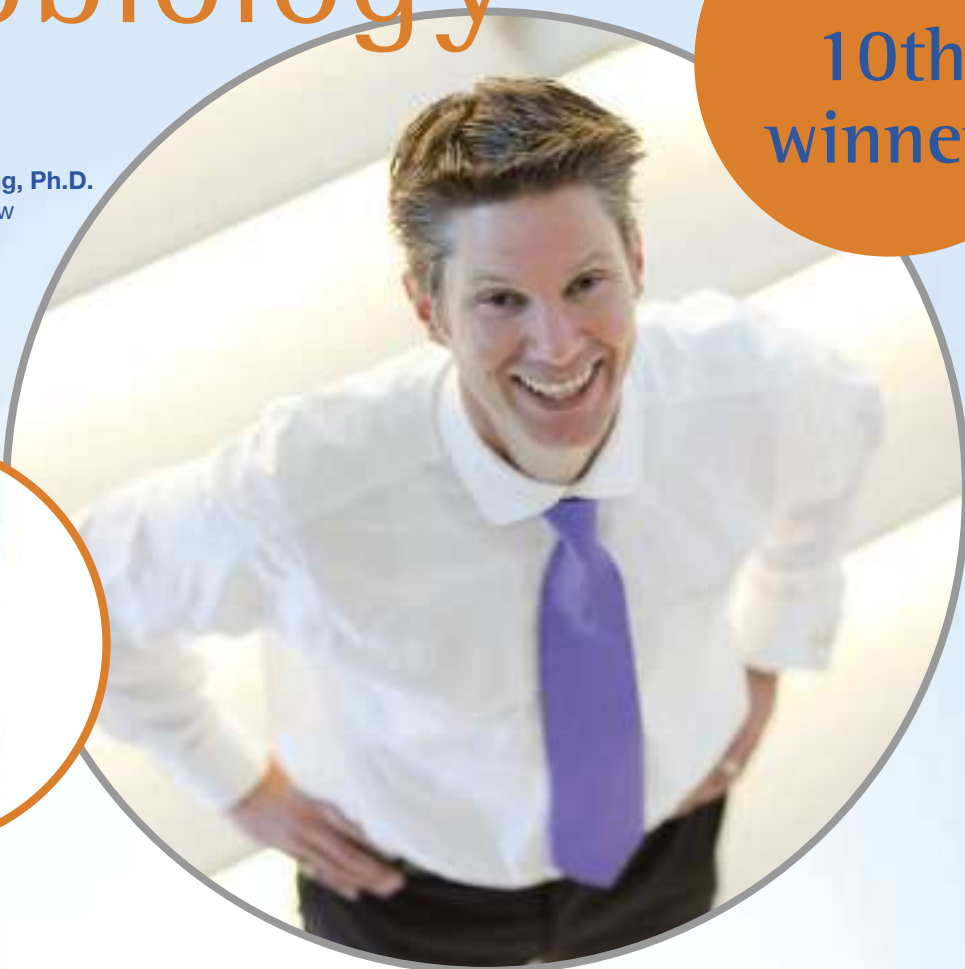
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